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BLOOD COAGULATION AND THROMBOSIS
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SURGERY OF THE HEART AND THORACIC BLOOD VESSELS
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Blood Coagulation and Thrombosis: Introduction

Research in blood coagulation, which for over a century moved at a strictly academic tempo, has recently acquired a remarkable acceleration. Part of this is due to what might be called a mutually catalytic reaction between interest and knowledge relating to the haemorrhagic states; as new knowledge of coagulation is gained, new aspects of these conditions are revealed, which in turn provide both the stimulus and the means for further investigation. A more important accelerating influence has been the introduction of anticoagulant therapy for thrombosis. This treatment has to be controlled by tests of blood-clotting function, and its widespread use has forced numberless pathologists to become involved in coagulation research in their efforts to standardize methods and understand their results. In consequence there has been a flood of new facts, a crumbling of comfortable old theories, and a sense of mounting confusion which can only be resolved by the rebuilding of a new and wider concept. Already the outlines of such a concept are becoming apparent, but they also emphasize the gaps in its structure.

This number of the *British Medical Bulletin* contains contributions describing some aspects of recent work on blood coagulation and its practical application in medicine. Only certain parts of the subject are

dealt with—a comprehensive account would require several volumes. Each article, however, has been written by an expert on the particular branch of the subject covered. Since the reader who is without special knowledge may find some of the terms and ideas confusing, it may be helpful to include in this introduction a brief description of their evolution.

The so-called " classical " theory of blood clotting will probably be familiar, and has been the basis of most of the work done during the past 50 years. According to this theory, the circulating blood contains two special proteins, prothrombin and fibrinogen, which remain unchanged so long as the blood is in contact only with normal vascular endothelium. When blood is shed, thus coming into contact with damaged tissues or a " foreign " surface, a third factor, thromboplastin, comes into play, with the co-operation of ionized calcium, to convert prothrombin to thrombin, which in turn converts fibrinogen to insoluble fibrin. Though this theory remained more or less unchallenged for half a century, it failed to explain many of the more recent observations, and for a time seemed likely to be swept away altogether. It is now apparent that almost all of the controversial findings become explicable by merely extending the classical theory, without altering its basic structure. This extension concerns the factor called thromboplastin. This was supposed by the classical theory to be mainly derived from damaged tissues, which have a powerful coagulant action; but, since blood taken by a clean venepuncture without contact with tissue still clots rapidly, it was also supposed that there must be some source of thromboplastic activity in the blood itself. The origin of this " intrinsic " thromboplastin was thought to be the platelets, which disrupt on contact with a foreign surface, though it was known that platelet extracts had only a feeble coagulant action. The major advance in coagulation research during the past few years has been the recognition of a whole system of new factors contained in the blood which react, probably in stages, to produce a short-lived but extremely powerful activator of prothrombin, called here thromboplastin. The time occupied by the thromboplastin-forming reactions accounts for most of the time needed for blood to clot, and it is the occurrence of faults in this system which accounts for most of the haemorrhagic states due to defective coagulation. The factors known to be concerned in the system are these: antihaemophilic globulin (AHG), Christmas factor, the platelets (and possibly plasma lipids), Factor V, Factor VII and calcium. Others have also been postulated (for example, Factor X and plasma thromboplastin antecedent (PTA)), but their existence is in doubt. The way in which these factors react is not known, but there is evidence to suggest that, following contact with a foreign surface, AHG, Christmas factor and the platelets react in the presence of calcium to form an intermediate product very similar in its coagulant action to tissue extract. This intermediate product reacts with Factors VII and V to form the direct activator of prothrombin. During these reactions, AHG and Factor V are consumed, but Factor VII and Christmas factor are not, and are thus found in serum. Defects in the thromboplastin-forming system produce important haemorrhagic states. A deficiency of AHG causes the bleeding of haemophilia; a deficiency of Christmas factor causes Christmas disease (or haemophilia B); Factor V deficiency is the cause of " parahaemophilia ", and Factor VII deficiency is the main result of the administration of the dicoumarin group of anticoagulant drugs.

At first sight, the unravelling of this system seems to have greatly increased the complexity of blood coagulation theory. In fact it has clarified matters, because the many observations which seemed to be completely discordant with the old classical theory can now be fitted into a relatively simple extension of it. From a practical point of view, the diagnosis and treatment of several important haemorrhagic states has been definitely advanced. Not only are we now in a better position to understand the clotting defects in the haemophilia-like states, but they can be separated into previously unrecognized groups, and the specific defects can be assessed quantitatively, thus providing for the first time a basis for treatment.

As so often happens, advances have taken place simultaneously in different centres throughout the world. In consequence there has arisen a confusion of multiple terminology which has reached almost ludicrous proportions, and is responsible for many of the difficulties which beset the workers on coagulation. There are now nine or ten different names for each of the newly recognized factors and many names which probably do not refer to real entities at all. Fortunately the situation has become so intolerable that an International Committee on the Nomenclature of Blood Clotting Factors is in the process of formation, which, when established, may succeed in reducing the existing chaos to a semblance of order. Meanwhile, in this number of the *British Medical Bulletin* no attempt has been made to use all the synonyms for all the factors that are mentioned.

In the section devoted to the mechanism of coagulation, K. Bailey & F. R. Bettelheim deal with the nature of the thrombin–fibrinogen reaction. This, the last link in the long chain of reactions, is the most important for several reasons: firstly because fibrin formation is, after all, presumably the main purpose of the clotting system; secondly because fibrin formation is the only observable change available to the coagulation worker, from which he has to infer all that he knows or thinks he knows about preceeding reactions; thirdly because it is only in this last reaction that even an inkling has been obtained of the chemical and physico-chemical changes involved in clotting. A description of the large-scale separation of human clotting factors for clinical use is given by R. A. Kekwick, who has been responsible for important developments in this field. R. Biggs, who, with A. S. Douglas, introduced the now widely used thromboplastin-generation test, discusses the interpretation and significance of methods for estimating the activity of factors and reactions in the clotting process. J. F. Ackroyd, in two articles, reviews the rapidly growing information on the functions of the platelets and their integration with clotting and vascular contraction in the complex mechanism of haemostasis, and also describes the causes and effects of platelet lysis or agglutination. W. R. Pitney & J. V. Dacie, who were concerned with the recognition of Christmas disease, present recent advances in the understanding of the haemophilia-like states, and C. Hougie discusses another group of coagulation defects, those due to the development of anticoagulant factors in the patient's blood.

It has been emphasized that there have been fruitful advances in knowledge of the clotting mechanism, and of the effects of its failure; but for every patient who dies as a result of deficient coagulation, there are thousands who die of thrombosis. Here we have a strange situation; a vast effort is being expended on the treatment of thrombosis with anticoagulants, but practically nothing is known of its causes. This disproportion is reflected in the articles in the section dealing with thrombosis. There are comprehensive and advanced accounts of the chemistry of heparin and its related compounds by K. W. Walton and of the coumarin anticoagulant drugs by R. B. Hunter & D. M. Shepherd; A. S. Douglas describes the recent views on the action of the coumarin drugs on the clotting system, and C. C. Burt reviews their clinical application. We do not know, however, how these drugs produce their beneficial effects, which cannot be explained simply by their depression of coagulation. The aetiology of thrombosis is discussed only by A. H. T. Robb-Smith, by J. F. Ackroyd, in passing, and by J. B. Duguid by implication. Professor Duguid presents evidence for his stimulating hypothesis that mural thrombosis is the origin rather than the result of atherosclerosis, a transposition of cause and effect which increases the importance of thrombosis but eliminates one of the few current ideas on its aetiology. A. H. T. Robb-Smith, in a refreshingly characteristic article, paints an historical picture of the subject on a wide canvas, which serves to emphasize the paucity of facts relating to thrombosis. He roundly scolds the coagulation workers for their preoccupation with artificial systems and for having neglected abnormally active, in favour of abnormally inactive, clotting. If I may take up the defence on this point, it would be fair to say that, in coagulation work, it is often the abnormal deficiency of a factor which reveals its normal existence. Hitherto our knowledge of clotting and the tests available have been too crude to distinguish any increase in the coagulability of the blood which may be responsible for thrombosis. Though there has not yet been time to apply the new knowledge or to devise new tests which might reveal such a change, there is now no lack of determination on the part of coagulation workers to study the causes of thrombosis; but are all the vascular occlusions usually labelled "thrombosis" primarily due to fibrin formation or to causes outside the scope of blood coagulation research?

Since the contributors to this number have, with admirable restraint, refrained from speculation, perhaps I might be permitted a little on my own account in this introduction. From the writings of J. N. Morris, it would seem that, though the incidence of coronary thrombosis (or perhaps more accurately, of myocardial ischaemia) has risen sharply during the past 40 years, the incidence of coronary atheroma has decreased. If this is so, and if atheroma is still regarded as an essential factor in coronary thrombosis, then some other influence must also be involved, one which has become more potent during recent years. This may be an increased coagulability of the blood, rendering more likely the occurrence of clotting in atheromatous vessels. Such increased coagulability might be limited to certain individuals, in which case it has escaped detection by the commonly used tests, but might now be demonstrable by modern methods; or there may have been a steady increase of coagulability affecting the majority of the population, which would not be easily detectable, since the past is not available for comparative investigation in this respect. In any case, if we postulate a recent change

in the incidence of thrombosis we should look for a recently operating cause. There is much talk of the "stress" of modern life as the root of modern ills, but specific phenomena usually have specific causes, and the idea of stress has always seemed rather nebulous. A short time before Sir Edward Mellanby discovered the astonishing toxicity of agenized flour, I was assured by an intelligent veterinary surgeon that the rising incidence of canine hysteria was due to the nervous strain of a modern dog's life. During recent years there has been a rapidly increasing use of chemical agents in food preparation, some of which, like agene, may have unsuspected effects. Many other by-products of progress have been blamed for new diseases, including atmospheric pollution, most of the commonly used drugs, *x* rays, and even the broadcast energy of radio transmitters! To test each of these for their possible implication in the problem of thrombosis would be a herculean task. But there is one pointer which might be worth following. There are indications that the liability to thrombosis is related to the number of fat particles usually present in the subject's plasma, and it has been shown by H. W. Fullerton and his colleagues and by J. C. F. Poole that an increase of plasma fat is associated with an increased coagulability of the blood. Since the human race has consumed fat for many generations, and the tendency to thrombosis has only recently increased, one can postulate either some recent change in the normal fat-transport system, or in the fat itself. There is in progress active work on the action of heparin in fat transport, which may throw light on the first possibility. Concerning the second, there has been a marked and recent change in the sort of fat we eat. Between 1913 and 1953 in Great Britain, the average daily intake of animal fat has risen only slightly from 83 g. to 87 g., but in the same period the daily intake of vegetable fat has increased from 5 g. to 41 g., this being mostly derived from ground nut, soya and palm oils, and consumed in the form of cooking fat and margarine. It is now known that different lipids have widely different effects on thromboplastin formation, some being active potentiators, others powerful inhibitors. I suggest that the effects of this change in diet should be investigated, since the ingestion of fat which is more actively coagulant might render an individual, who has both a naturally high plasma-fat level and atheroma, more liable to thrombosis than he would have been 40 years ago.

R. G. Macfarlane

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ASSESSMENT OF CLOTTING EFFICIENCY

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1 Definition of the coagulation defect
  a Fibrin formation, fibrinopenia and antithrombin
  b The one-stage " prothrombin " time (one-stage test) and thrombin formation
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The difficulty of work on blood coagulation can be traced to a lack of definition in the techniques used. It is seldom possible to know what is measured by a technique or how reliable a measure it gives, or even what it is desirable that it should measure. This lack of clarity is inherent in the problem. Coagulation is the only observable phenomenon which culminates a long chain of preliminary reactions initiated by contact with a foreign surface, leading in series to thromboplastin formation, the conversion of prothrombin to thrombin and finally to clotting. There are at least eight primary substances involved in these reactions and probably more than three intermediate products are formed. All of these substances can be studied only by their effect on the final clotting of fibrinogen. Very little is known about the relative contributions of the various factors to the over-all reaction and were it possible reliably to assay each single factor by an independent method the problem might still be far from solution. It is probable that techniques which study reactions as a whole are more informative than specific assay methods.

The majority of techniques used to study blood coagulation have been evolved more or less empirically for the study of particular diseases and these techniques in the main do study reactions rather than substances. Their interpretation naturally depends on the extent of knowledge at a particular time and, for their intelligent application, it is important that they should be understood in terms of the most recent views of the clotting process.

The earliest technique used consists of putting whole blood in a capillary or test-tube and observing the time which elapses before clotting occurs. This whole-blood clotting time is by far the simplest test and it might seem logical in describing tests of clotting function to start with the simplest and progress to the more elaborate. But with advancing knowledge the simplest tests are not always the easiest to interpret and it has been found more convenient to discuss the techniques in their application to specific coagulation defects. The use of the techniques is therefore described from two main points of view: the definition of the clotting defect, and the assessment of the severity of the defect in relation to haemostasis. The definition of a clotting defect implies some knowledge of the recent theories of blood coagulation. A summary of this aspect is given in Dr R. G. Macfarlane's Introduction to this number of the Bulletin. Two tables which may help in the understanding of techniques are included in this communication. Table I gives the better-recognized coagulation factors and some reagents, their modern synonyms and mode of action, and their clinical deficiencies. Table II presents a hypothetical scheme of the probable stages of blood coagulation.

1. Definition of the Coagulation Defect

For clarity the coagulation process has been arbitrarily divided into a number of stages which in operation naturally overlap and grade one into the other (Table II). These subdivisions are thromboplastin formation, thrombin formation and fibrin formation. For convenience thromboplastin formation has been divided into three phases: the first involving activation of various factors by contact with a foreign surface (stage 1), the early stages of thromboplastin formation (stage 2), and the later stages, in which Factors V and VII are involved (stage 3). These distinctions are helpful both because the recognized clotting defects influence one or other of these phases most markedly and because the tests of clotting function record abnormalities in the different stages of clotting. In dealing with this problem, I propose to work backwards through the scheme in Table II, starting with defects in fibrin formation and progressing to defects in thrombin or thromboplastin formation. This reverse order of procedure is necessary because all of the techniques are based on observations on fibrin formation and the certainty of information decreases with separation from this stage.

a. Fibrin Formation, Fibrinopenia and Antithrombin

When a strong solution of thrombin is added to normal plasma a solid fibrin clot appears almost instantaneously. The amount of this clot can be estimated quantitatively by a number of well-established techniques (Biggs & Macfarlane, 1953). In some patients fibrinogen is reduced in amount, causing a bleeding tendency, and this reduction can readily be verified.

When an excess of an anticoagulant which interferes with the thrombin-fibrinogen reaction, such as heparin, is present in the blood, the reaction to a strong solution of thrombin may be nearly normal. When tested with a series of weaker solutions, the abnormality may be detected by an abnormal lengthening of the clotting time in the high dilutions.

b. The One-Stage " Prothrombin " Time (One-Stage Test) and Thrombin Formation

During normal clotting, thrombin is formed from a precursor substance—prothrombin. According to the classical theory of blood coagulation this change is brought about directly by an enzyme, thromboplastin, in the presence of

TABLE I. SOME COAGULATION FACTORS AND REAGENTS, THEIR PROPERTIES AND RECOGNIZED CLINICAL DEFICIENCIES

Reagent or coagulation factor	Contemporary synonyms	Properties	Recognized deficiency
Fibrin	—	Product of the reaction of thrombin on fibrinogen	—
Fibrinogen . . .	—	Protein fraction precipitated by 33% saturation with $(NH_4)_2SO_4$ Clotted by thrombin	Essential fibrinopenia (rare) Severe liver disease
Thrombin . . .	—	Coagulant of fibrinogen formed from prothrombin during clotting	—
Prothrombin . . .	—	Precursor of thrombin Adsorbed to $Al(OH)_3$	Essential hypoprothrombinaemia (rare) Reduced by dicoumarin drugs, in liver disease, in vitamin K deficiency, and in haemorrhagic disease of the new-born
Factor V (Owren, 1947)	Ac-globulin (Ware, Guest & Seegers, 1947) Pro-accelerin (Owren, 1951) Labile factor (Quick, 1943)	Component required for thromboplastin formation. Not adsorbed to $Al(OH)_3$. Absent from serum	Essential deficiency often classed with "hypoprothrombinaemia" from the results of the one-stage test Reduced in liver disease, occasionally in acute infections and secondary carcinoma
Factor VII (Koller, Loeliger & Duckert, 1951)	Pro-convertin (Owren, 1951) Co-thromboplastin (Mann, 1949) Serum prothrombin conversion accelerator (SPCA) (Alexander, de Vries & Goldstein, 1949a) Serum Ac-globulin (Ware & Seegers, 1948a) Stable factor (Stefanini, 1953)	Component required for thromboplastin formation. Adsorbed to $Al(OH)_3$. Present in serum	Essential deficiency often classed with "hypoprothrombinaemia" from the results of the one-stage test Reduced in liver disease, in vitamin K deficiency and in haemorrhagic disease of the new-born. Reduced by dicoumarin drugs
Antihaemophilic globulin (AHG)	Antihaemophilic globulin A (von Cramer, Matter & Loeliger, 1953)	Component required for thromboplastin formation. Not adsorbed to $Al(OH)_3$. Absent from serum	Haemophilia
Christmas factor (Biggs *et al.* 1952)	Plasma thromboplastin component (PTC) (Aggeler *et al.* 1952) Antihaemophilic globulin B (von Cramer *et al.* 1953)	Component required for thromboplastin formation. Adsorbed to $Al(OH)_3$. Present in serum	Christmas disease (PTC deficiency)
Normal serum . . .	—	Contains Factor VII and the Christmas factor but not Factor V, antihaemophilic globulin or prothrombin	—
$Al(OH)_3$-treated plasma	—	Contains Factor V and antihaemophilic globulin but not Factor VII, Christmas factor or prothrombin	—

calcium chloride; this enzyme was thought originally to be provided by tissue extracts. This theory may be expressed:

$$\text{prothrombin} \xrightarrow[CaCl_2]{\text{thromboplastin}} \text{thrombin}$$

$$\text{fibrinogen} \xrightarrow{\text{thrombin}} \text{fibrin}$$

Since the clotting time of fibrinogen is inversely proportional to the concentration of thrombin, the clotting time of a solution containing prothrombin, fibrinogen, thromboplastin and calcium will give a measure of its prothrombin content, if it is assumed that prothrombin is converted to thrombin at a uniformly rapid speed. This theoretical reasoning formed the original basis for the interpretation of the one-stage prothrombin time test, which was thought to give a quantitative measure of prothrombin (Quick, 1935, 1942).

In this test 0.1 ml. of brain extract and 0.1 ml. of $CaCl_2$ are added to 0.1 ml. of plasma and the clotting time is recorded. The test is carried out with normal and abnormal plasma and the clotting times (being assumed to be proportional to the prothrombin concentration) can be compared. This test is undoubtedly the most useful single test of clotting function ever devised and by its use it has been possible to study vitamin K deficiency, haemorrhagic disease of the newborn, and liver disease, and to use anticoagulants of the dicoumarin group in the treatment of thrombosis.

TABLE II. A HYPOTHETICAL SCHEME OF BLOOD COAGULATION, WITH AN INDICATION AS TO THE STAGES OF THE MECHANISM WHICH THE VARIOUS TESTS OF CLOTTING FUNCTION MEASURE

Clotting tests	Arbitrary stage of clotting	Probable reactions involved	Time occupied by the reactions
Whole-blood clotting time and thrombin-generation test; Prothrombin-consumption test; Thromboplastin-generation test	Thromboplastin formation		
	Stage 1: The effects of contact with a foreign surface	Nature of the reactions unknown	
	Stage 2: The early stages of thromboplastin formation	Platelets, Christmas factor and anti-haemophilic globulin (AHG) ↓ $CaCl_2$ Intermediate product or products probably equivalent to tissue "thromboplastin"	2–5 min.
One-stage "prothrombin" time	Stage 3: Completion of thromboplastin formation	+ Factors V and VII ↓ $CaCl_2$ Thromboplastin	8–10 sec.
Prothrombin assay	Thrombin formation	+ Prothrombin ↓ $CaCl_2$ Thrombin	2–5 sec.
Tests of fibrin formation	Fibrin formation	+ Fibrinogen ↓ Fibrin	2–5 sec.

The test as originally interpreted was based on one very important assumption—that the speed of prothrombin conversion in the presence of tissue extract is uniform in all plasma samples—and it is now widely recognized that this assumption is not justified. It is now clear that the speed of prothrombin conversion is affected by two factors, called Factors V and VII (Table I). The classical theory must be expanded to include them (Biggs, Douglas & Macfarlane, 1953b):

$$\text{brain extract} + \text{Factor V} \xrightarrow[CaCl_2]{\text{Factor VII}} \text{thromboplastin}$$

$$\text{prothrombin} \xrightarrow[CaCl_2]{\text{thromboplastin}} \text{thrombin}$$

$$\text{fibrinogen} \xrightarrow{\text{thrombin}} \text{fibrin}$$

The one-stage prothrombin time will record an abnormality in any of these reactions, which by reference to Table II will be seen to include stage 3 of thromboplastin formation. Some difficulty in terminology arises. If the term "thromboplastin" is retained for the direct activator of prothrombin, tissue extracts provide an intermediate product of thromboplastin formation rather than thromboplastin itself.

Deficiencies of prothrombin, Factor V, and Factor VII can quite readily be distinguished from each other. From the properties of Factor V (Table I) it will be seen that Factor V will be present in $Al(OH)_3$-treated plasma from which Factor VII and prothrombin are absent. Thus the addition of 10% of $Al(OH)_3$-treated plasma to the patient's plasma will largely correct the abnormality as tested by the one-stage technique, if Factor V is deficient. Similarly Factor VII deficiency will be corrected by the addition of 10% of normal serum. Alternatively, Factor V and VII deficiency can be measured more quantitatively by the methods of Quick & Stefanini (1948) and Ware & Seegers (1948b) (Factor V), or of Koller, Loeliger & Duckert (1951) and Owren & Aas (1951) (Factor VII).

A difficulty sometimes arises in the diagnosis of prothrombin deficiency because prothrombin deficiency by itself may not cause much lengthening of the one-stage prothrombin time (Biggs & Douglas, 1953a). Prothrombin deficiency can be recorded quantitatively by the two-stage procedures such as that of Ware & Seegers (1949) or by the simpler method of Biggs & Douglas (1953a).

c. The Thromboplastin-Generation Test, and Defects in Stage 2 of Thromboplastin Formation

Brain extract used in the previous tests is an artificial reagent but it is probable that during clotting a substance of activity resembling that of tissue extracts is formed. This hypothetical substance probably arises during the formation of a powerful blood thromboplastin which can be demonstrated by the thromboplastin-generation test. (See Biggs & Douglas, 1953b.)

The factors thought to be necessary for blood thromboplastin formation are antihaemophilic globulin and Factor V (both contained in $Al(OH)_3$-treated normal plasma), Factor VII and the Christmas factor (both contained in normal serum) and platelets (see Tables I and II). In the thromboplastin-generation test these reagents are prepared from normal blood; when they are mixed with $CaCl_2$ and incubated at 37° C. a very powerful thromboplastin forms in the mixture. The potency of this thromboplastin can be assessed at intervals during its formation by recording its ability to clot plasma or a mixture of prothrombin and fibrinogen.

The blood of patients with defects in the early stages of blood thromboplastin formation will have a normal one-stage

prothrombin time because a tissue extract, which replaces the product of these early reactions, is added to the blood in this test. Haemophilia and Christmas disease (Biggs, Douglas, Macfarlane, Dacie, Pitney, Merskey & O'Brien, 1952; Aggeler, White, Glendening, Page, Leake & Bates, 1952) are two closely related conditions which are clinically indistinguishable; both are caused by defects in stage 2 of thromboplastin formation (Table II), but are due to the lack of different substances.

If, in the thromboplastin-generation test, the normal $Al(OH)_3$-treated plasma is replaced by that from a haemophilic subject, the formation of thromboplastin is much reduced, this reduction being due to deficiency of antihaemophilic globulin in the system. Similarly, in Christmas disease, if the normal serum is replaced by serum from the patient, thromboplastin formation is reduced, this reduction being due to the absence from the patient's serum of the Christmas factor. Thus the thromboplastin-generation test can be used to distinguish between patients with haemophilia and those with Christmas disease.[1]

Certain patients develop anticoagulants in their blood which interfere with normal thromboplastin formation. The presence of the anticoagulant can be demonstrated by using the thromboplastin-generation test (Biggs & Douglas, 1953b).

d. Tests which Include the Earliest Stages in Coagulation

The tests so far described involve the addition of coagulants (thrombin or tissue extracts) to plasma, or the preparation of reagents from citrated blood or serum. It is probable that the earliest stages of clotting involve activation of the Christmas factor and some alteration in platelets (stages 1, 2 of Table II; see also Biggs, Douglas & Macfarlane, 1953a). These changes, which may be interdependent, are brought about in some unexplained manner by contact with a foreign surface. In the body this surface is probably provided by damaged tissue, in vitro the glass surface provides the contact stimulus.

The tests so far described do not attempt to record abnormalities of this phase, for the study of which whole untreated blood must be used. Three tests are available: these are the estimation of the whole-blood clotting time (by the Lee and White method; see Lee & White, 1913), the prothrombin-consumption test, and the thrombin-generation test.

i. *The whole-blood clotting time (Lee & White, 1913; Biggs & Macfarlane, 1953).* In the whole-blood clotting time test, 1 ml. of blood is put into each of four glass tubes. Then the tubes are incubated at 37° C. and tilted at intervals until the blood solidifies, the time that elapses between collection of the blood and its clotting being recorded. The test is of course entirely non-specific, for any coagulation defect will cause a lengthening of the clotting time if the defect is sufficiently severe. The test is extremely simple and, in conjunction with tests to define the clotting abnormality, it is useful.

ii. *The prothrombin-consumption test (Soulier, 1948; Quick & Favre-Gilly, 1949; Merskey, 1950).* When whole blood clots in a glass tube practically all of the prothrombin is converted to thrombin. This conversion is brought about by the blood thromboplastin normally formed during clotting. If there is any gross abnormality in thromboplastin formation the conversion of prothrombin to thrombin will be delayed or absent. The methods used for the assessment of prothrombin consumption depend on comparing the coagulant ability of plasma and serum, on the addition of tissue extract and $CaCl_2$, at a determined time after coagulation has occurred. All of the methods used routinely are empirical in that no serious attempt is made to obtain a true measure of prothrombin.

iii. *The thrombin-generation test (Macfarlane & Biggs, 1953).* Carried out on whole blood, this test consists in placing 2 ml. of blood in a tube at 37° C. and in removing samples at intervals and testing the thrombin formed by addition of these samples to fibrinogen, the clotting time of which is recorded. In this way a curve relating thrombin concentration to time can be drawn. The test is carried out on normal blood and on that of the patient, and the two curves compared. The results have the disadvantage that they cannot be expressed quantitatively. On the other hand the test detects small alterations from normal and gives a good picture of the general pattern of thrombin formation.

2. Significance of Tests of Clotting Function

Although the exact mechanism of clotting is unknown, a provisional hypothesis of the sort of reactions that occur can be formulated (Table II). This scheme makes no attempt to analyse the process in detail but it gives a reasonable view of the complexity of the process and the approximate order in which the reactions occur; moreover some deductions can be made about the time occupied by the various stages. The initial stages (stages 1 and 2), which involve contact with a foreign surface and the formation of thromboplastin, are by far the most time-consuming, occupying from two to five minutes. Subsequent reactions require only a few seconds.

In the light of a hypothesis of this sort the interpretation of tests of clotting function is possible. The whole-blood clotting time and thrombin-generation test record abnormalities at any stage of the process but, since the first two are the most time-consuming, abnormalities in these are likely to have the greatest effect. The results of the two tests differ from each other quantitatively and qualitatively, the thrombin-generation test giving more information and being far more sensitive to minor defects; unfortunately it is technically more difficult to perform.

The thromboplastin-generation test records the efficiency of thromboplastin formation but the changes associated with contact are not included. The test can be used to differentiate between deficiency of the factors concerned in stages 2 and 3 of the clotting process. The prothrombin-consumption test gives similar information, though it is less specific and less sensitive to minor deficiencies.

The one-stage prothrombin time test measures a terminal part of the thromboplastin-forming reactions and all reactions which follow. Clotting times by this technique are normally short because these phases of clotting are rapid. The defects recorded in this phase affect the whole-blood clotting time only if they are very marked.

Other tests modified to give a quantitative measure of factors provide different information; with the exception of the prothrombin assay, they are not concerned with the efficiency of reactions as a whole.

3. Clotting Efficiency and Haemostasis

The extent to which a clotting abnormality affects haemostasis can be assessed roughly in terms of the available tests,

[1] See Pitney & Dacie, p. 11 of this number of the Bulletin.—Ed.

the level at which bleeding is likely having been worked out empirically in each condition.

a. Fibrinogen Deficiency

Deficiency in fibrinogen is rare. From the work of Pinniger & Prunty (1946), Stefanini & Petrillo (1949) and Alexander, de Vries & Goldstein (1949b), it appears that clotting efficiency changes significantly when the fibrinogen concentration is diminished to 60 mg./100 ml. Exceptionally, however, patients whose clotting defect has been attributed to fibrinogen deficiency have been found to have fibrinogen levels greater than 100 mg./100 ml. (110 mg., Allibone & Baar, 1943; Heinild, 1944), and we have observed a patient with a very poor efficiency of fibrin formation whose fibrinogen level was estimated at 100 mg./100 ml.

b. The One-Stage Prothrombin Time Test and Clotting Efficiency

The abnormalities which cause a prolonged one-stage prothrombin time when the fibrinogen level is normal are deficiencies of Factors V and VII and of prothrombin. In most clinically important conditions there is not a single deficiency of one of these factors but two or more are affected together (Table I; and see also Biggs & Macfarlane, 1953). In individual cases one factor may be more affected than another. The great advantage of the one-stage prothrombin test is that it records the total efficiency of the reactions involving all these factors and with a few rare exceptions gives a good over-all picture of the various contributions of the different factors to the clotting efficiency. In general it can be said that, if the one-stage clotting time exceeds the normal by a factor of three, then the danger of haemorrhage exists. Many patients may have a clotting time exceeding this level without bleeding, but below this level bleeding is rare.

The exceptional subjects in whom the one-stage test may give misleading results are those who have prothrombin deficiency as a single defect. These patients may have a relatively normal one-stage prothrombin time (Biggs & Douglas, 1953a), but a haemorrhagic tendency may exist. It is probable that a prothrombin concentration of less than 10 % as measured by a two-stage technique would give rise to a haemorrhagic tendency.

c. Haemophilia

Of the deficiencies in the early stages of thromboplastin formation, by far the most important is haemophilia or antihaemophilic globulin deficiency. In this condition the level at which haemorrhage is to be expected can be deduced in relation to various tests of clotting function, because a very wide range of cases of differing severity is available. The majority of haemophilic patients have a long whole-blood clotting time as tested by the Lee and White method, the prothrombin-consumption test gives abnormal results, no thrombin is detected in the thrombin-generation test, and the thromboplastin-generation test records negligible amounts of antihaemophilic globulin. These patients are severely affected clinically with numerous spontaneous haemorrhagic episodes. At the other extreme are patients whose whole-blood clotting time and prothrombin consumption are within normal limits. On the whole these patients are less severely affected, and bleed only if they are injured. Brinkhous, Langdell, Penick, Graham & Wagner (1954) record a patient with an antihaemophilic globulin level of 16 %. This patient had a normal whole-blood clotting time and prothrombin-consumption test, he led a normal life but bled excessively after teeth extractions. A patient observed by us had about 7.5 % of antihaemophilic globulin; he had severe trouble with bleeding after dental extractions and suffered from a marked bruising tendency but had no haemarthroses. This patient had a normal whole-blood clotting time but the prothrombin-consumption index was at the upper limits of the normal range.

These results suggest that neither the whole-blood clotting time nor the prothrombin-consumption index are adequate tests of clotting efficiency in haemophilia, since a bleeding tendency may exist when these tests record normal results. The tests naturally remain useful in diagnosis because the majority of haemophilic patients are very severely affected. In general it is probable that the clotting time of a haemophilic patient will be normal if more than 5 % of antihaemophilic globulin is present. The prothrombin-consumption index will be normal with levels of more than 10 % of antihaemophilic globulin. For surgery to be undertaken with reasonable safety, the level must rise substantially above 20 %, probably to near 50 % of normal, and must remain at this level or be returned to this level at frequent intervals until healing is well advanced. Since antihaemophilic globulin disappears rapidly from the blood of a haemophilic patient this means, at present, the transfusion of 500–1,000 ml. of fresh plasma at intervals of 24–48 hours.

To assess safety for surgery some direct assay of antihaemophilic globulin is required. This assay can be achieved by recording the ability of the patient's blood to correct the defect in an untreated and severely affected haemophilic patient (Merskey, 1951; Graham, Collins, Godwin & Brinkhous, 1951). Alternatively, the thromboplastin-generation test or the thrombin-generation test may be used. Both of these tests are more sensitive than either the clotting-time or prothrombin-consumption tests, and they will probably record levels of antihaemophilic globulin up to or exceeding 50 % of normal.

d. Christmas Disease

In Christmas disease it is probable that the same general principles apply. Patients who are mildly affected may have a normal whole-blood clotting time and prothrombin-consumption index though they bleed excessively on injury. In the treatment of these patients also, large transfusions may be necessary to control bleeding.[2]

4. Conclusion

The usefulness of a test of clotting function is not necessarily related to its ability to give a reliable quantitative measure of a particular substance. The one-stage prothrombin time test, thought initially to give a specific measure of prothrombin, is more rather than less useful because it is sensitive to several different types of abnormality. The wider range of the test makes it possible, by simple modification, to detect deficiencies of three factors rather than of one. Similarly the thromboplastin-generation test is not in itself a test of any particular substance, but it can be adapted to distinguish between five or six recognized abnormalities; in practice its main usefulness lies in the study of haemophilia, Christmas disease and the circulating anticoagulants.

[2] See Pitney & Dacie, p. 11 of this number of the Bulletin.—Ed.

The usefulness of a test depends on knowing the stage of clotting that it can be used to study and the sensitivity of the test in relation to the patient's tendency to bleed. The one-stage prothrombin time test, for example, is quite sufficiently sensitive to measure clinically important clotting defects, and, in relation to the scheme in Table II, its range is well defined. If it is desired to record abnormality of the blood in its reaction to surface contact, then neither the one-stage prothrombin time nor the thromboplastin-generation test will supply the necessary information. Tests such as the whole-blood clotting time, the prothrombin-consumption test or the thrombin-generation test must be used. In this way an intelligent use of a few technically simple tests can be employed to define coagulation abnormalities and to assess their severity.

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HAEMOPHILIA AND ALLIED DISORDERS OF BLOOD COAGULATION

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Until two years ago haemophilia was considered a single entity. The disease was diagnosed with confidence on the clinical history of the patient, aided by simple laboratory tests. A male with an abnormal tendency to bleeding since infancy or childhood was usually assumed to be suffering from haemophilia if there was a history of similar occurrences in other male members of his family. The abnormal laboratory findings which were considered pathognomic of the disease were a prolonged whole-blood coagulation time and a diminished consumption of prothrombin. Characteristically, too, small amounts of normal plasma added in vitro could be shown to restore to normal the prolonged coagulation time of haemophilic blood. The results of other tests commonly used in the investigation of haemorrhagic disorders, such as the prothrombin time, platelet count, bleeding time and plasma-fibrinogen estimation, were typically normal. The inheritance pattern of haemophilia, with its incidence almost exclusively in males, but with inheritance through clinically normal females, was thought to be absolutely characteristic.

It was concluded that the coagulation defect in haemophilia was due to an imperfect production of plasma thromboplastin and that this was the result of a deficiency of a plasma thromboplastin-precursor substance, generally referred to as antihaemophilic globulin (AHG). The abnormally slow formation of thromboplastin was considered to result in a slow and imperfect conversion of prothrombin to thrombin, and therefore to a prolongation of the whole-blood coagulation time. Other factors in the coagulation mechanism, such as platelets, prothrombin, Factors V and VII, and fibrinogen, could be shown to be normal, and usually a circulating anticoagulant could not be demonstrated.

1. Recent Advances in Knowledge

The comparatively simple concept of haemophilia outlined above was disturbed from time to time by observations which could not be easily explained. For instance, it was found, when using concentrated preparations of the antihaemophilic factor (Cohn's fraction I), that while some patients responded favourably, others were refractory although they presented an apparently similar clinical and pathological picture. Pavlovsky (1947) described an experiment in which a " haemophilic patient " was transfused with blood from another " haemophiliac ". He observed that the recipient's coagulation time was reduced to normal, an observation difficult to explain on the basis that both patients were suffering from the same deficiency. Koller, Krüsi & Luchsinger (1950) also observed that when samples of the blood of two " haemophiliacs " were mixed together the clotting time of the mixture became normal.

Aggeler, White, Glendening, Page, Leake & Bates (1952) investigated a male patient previously considered to have haemophilia. The bleeding tendency was shown to be due to a deficiency of a previously unrecognized clotting factor, also seemingly necessary for the production of plasma thromboplastin. This new factor, which Aggeler and his co-workers named plasma thromboplastin component (PTC), was shown to be easily separated from AHG by physico-chemical methods. The patient, who was a youth aged 16, was suffering from a condition clinically indistinguishable from haemophilia, except that there was no family history. The usual laboratory tests supported this diagnosis. It was by means of mixing experiments that the distinction from haemophilia was made. PTC was shown to be present in haemophilic plasma and also in normal plasma and serum. The defect in the patient's blood could be corrected by the addition of haemophilic plasma, but not by Cohn's fraction I.

Concurrently and independently, several patients were being studied in England and South Africa, and observations similar to those of Aggeler were made (Biggs, Douglas, Macfarlane, Dacie, Pitney, Merskey & O'Brien, 1952). By employing the thromboplastin-generation test (Biggs & Douglas, 1953), the AHG content of the blood of this series of patients was found to be normal. The factor which was deficient was referred to as the " Christmas factor ", from the name of the first patient studied in detail. It is apparent that PTC and the Christmas factor are the same substance. Biggs and co-workers also concluded tentatively that the inheritance of this second form of haemophilia might be identical with that of the classical form. When the thromboplastin-generation test was used as a means of diagnosis, seven patients out of 50 " haemophiliacs " were found to be suffering from the second form of the disease. These observations have been confirmed subsequently by workers from many parts of the world.

Shortly after the studies mentioned above, a third possible type of " haemophilia " was recognized. Rosenthal, Dreskin & Rosenthal (1953) described a haemorrhagic disorder which they considered was caused by deficiency of a third plasma

thromboplastin component; this they named plasma thromboplastin antecedent (PTA). It could be shown on the basis of mixing experiments that PTA-deficient blood corrected the coagulation abnormality of both the other two forms of haemophilia. The converse was also true.

2. Terminology of Haemophilia and Allied Disorders

It is unfortunately true that differences of opinion concerning nomenclature have caused much confusion. At a time when haemophilia was thought to be a single clinical and pathological entity, the name presented no difficulty. Now it is apparent that at least three inherited conditions—to say nothing of possible acquired deficiencies—are covered by this term. Aggeler and co-workers (1952) in their original publication used the term PTC deficiency. Subsequently they referred to the second form of haemophilia as deuterohaemophilia or plasma thromboplastin factor B (PTF-B) deficiency (Aggeler, White & Spaet, 1954). Biggs and her colleagues (1952) and Biggs, Douglas & Macfarlane (1953a) used the term Christmas disease for the second form. They considered that although clinically the disease might be identical with haemophilia, yet, as its pathogenesis was distinct, a complete break from the term haemophilia should be made. Graham & Brinkhous (1953) suggested that the term haemophilia should not be retained as a generic name for the whole group in which there is a defect in thromboplastin production, but should be used to denote solely the commonest disorder—AHG deficiency. The syndromes due to deficiency of blood-clotting factors other than AHG were referred to as " haemophilioid states ". By their classification, Christmas disease (PTC deficiency) would be called haemophilioid-state C, and PTA deficiency haemophilioid-state D. European workers, on the other hand, have used the terms haemophilia A for AHG deficiency and haemophilia B for Christmas disease (Soulier & Larrieu, 1953; Cramer, Flückiger, Gasser, Koller, Loeliger & Matter, 1953). Wiener (1953) suggested haemophilia I and II, and Koller (1954b) suggested Factor-VIII and -IX deficiency for AHG and Christmas-factor deficiency, respectively.

Obviously there is danger of misinterpretation in the many different names suggested for these diseases. For instance, at least nine names have been suggested for the condition described by Aggeler and colleagues as PTC deficiency, and by Biggs and her co-workers as Christmas disease, e.g., deuterohaemophilia (Aggeler, White & Spaet, 1954), haemophilia 2 (Wiener, 1953), haemophilia B (Soulier & Larrieu, 1953), haemophilioid-state C (Graham & Brinkhous, 1953), platelet-co-factor-2 deficiency (Seegers, 1954), β-prothromboplastin deficiency (Fantl & Sawers, 1954) and Factor-IX deficiency (Koller, 1954b)! We ourselves now feel that the two common types of haemophilia have so many features in common both clinically and genetically that the terms haemophilia A and B will prove to be the most widely acceptable. The two factors whose deficiency causes the syndrome are so dissimilar that quite distinct terms for them should be chosen. To call a factor PTC or PTF (A or B) and another PTA leads to complete confusion. There is certainly no reason to discard the term AHG; whether the term Christmas factor should be supplanted by another needs further consideration. However, as the term Christmas factor is widely used in British literature, we are retaining it, and until some international nomenclature is agreed upon, we will continue to use the term Christmas disease instead of haemophilia B for Christmas-factor deficiency.

3. Properties of the Thromboplastin-Precursor Substances

We shall not attempt to describe in detail the modern conception of the coagulation of blood in vitro. However, a brief description of the properties and possible functions of the three thromboplastin-precursor substances in the plasma will be given.

a. Antihaemophilic Globulin (AHG)

The exact chemical constitution of AHG is not known. However, when plasma is subjected to electrophoresis or is fractionated by chemical precipitation methods, AHG is closely associated with fibrinogen. It is thus probably a protein. It is precipitated by 25% saturation with ammonium sulphate. It is unstable on storage, and is not readily adsorbed by adsorbents such as aluminium hydroxide. It disappears from the blood almost completely during coagulation, and the usual tests do not reveal its presence in serum. It is present in normal amounts in the plasma of patients suffering from Christmas disease. Its concentration in ox plasma is about 16 times that in human plasma, and ox-blood AHG is able to replace human AHG in the coagulation of human blood (Macfarlane, Biggs & Bidwell, 1954).

b. Christmas Factor

This substance is activated during the coagulation of blood, and is more readily demonstrated in serum than in plasma. Electrophoresis of serum shows that the factor has the electrophoretic mobility of β_2-globulin (Aggeler, Spaet & Emery, 1954). It is precipitated from normal plasma by 33–50% saturation with ammonium sulphate. It is adsorbed by the prothrombin adsorbents, including aluminium hydroxide, barium sulphate and Seitz filters. It is stable on storage, but it is thermolabile, being destroyed by heating at 56° C. for 10 minutes. It is present in normal amounts in haemophilic blood. In many of its properties, Christmas factor resembles Factor VII.

c. Plasma Thromboplastin Antecedent (PTA)

Little is known of the properties of PTA. Judged by the reactions of the blood of patients suffering from PTA deficiency, the factor is normally present in both plasma and serum. It persists in stored plasma (R. L. Rosenthal, 1954) and is not adsorbed by barium sulphate (Rosenthal *et al.* 1953).

A comparison of the properties of these factors is shown in Table I.

TABLE I. PROPERTIES OF ANTIHAEMOPHILIC GLOBULIN (AHG), CHRISTMAS FACTOR AND PLASMA THROMBOPLASTIN ANTECEDENT (PTA)

Properties	AHG	Christmas factor	PTA
Stability on storage	Unstable	Stable	Stable
Adsorption to inorganic adsorbents	Not adsorbed	Adsorbed	Not adsorbed
Ammonium sulphate fractionation	Precipitated by 25% saturation	Precipitated by 33–50% saturation	Not known
Electrophoretic fractionation	In fibrinogen fraction	In β-globulin fraction	Not known
Presence in normal serum	Not demonstrable	Activity enhanced	Present

4. Role and Interrelationship of Antihaemophilic Globulin (AHG), Christmas Factor, and Plasma Thromboplastin Antecedent (PTA)

These three factors all appear to be intimately concerned with the production of plasma thromboplastin. In the haemorrhagic states caused by deficiency of these factors, there is imperfect conversion of prothrombin during coagulation in vitro. In the presence of added tissue thromboplastin, however, conversion of prothrombin proceeds in a normal manner. The formation of plasma thromboplastin can be readily studied by means of the thromboplastin-generation test (Biggs & Douglas, 1953; Biggs, Douglas & Macfarlane, 1953b). The factors known to be necessary for thromboplastin formation include platelets, antihaemophilic globulin, Christmas factor, Factors V and VII, and calcium. PTA is not included in this list, as little is known of its mode of action, and we have not had the opportunity of examining PTA-deficient blood, using the thromboplastin-generation test. Exactly how these many components interact to produce plasma thromboplastin is not clear. Bergsagel (1955) suggests that the Christmas factor combines with calcium to form an activated complex during an early stage of blood coagulation. There is some evidence that Christmas factor normally exists in plasma in combination with an inhibitor. AHG may release Christmas factor from this binding (Biggs & Macfarlane, personal communication, 1954). It is probable that several reactions of a complex nature take place in drawn blood before the formation of plasma thromboplastin. These take up most of the prolonged latent period of 5–10 minutes that elapses before visible clotting ensues. Once thromboplastin has been formed, the subsequent stages in the blood coagulation mechanism proceed rapidly, and are probably completed in about 10 seconds.[1]

5. Relative Incidence of Thromboplastin-Precursor Deficiency States

Many cases of Christmas disease have by now been described in the literature (see references). By adding recent cases of our own to the published figures, we have been able to obtain records of 70 patients in whom the laboratory findings have been characteristic—this figure does not include probable examples of the disease in affected relatives who have not been examined. Fifty-one of the patients have been discovered by re-examining 330 patients known to suffer from " haemophilia ". It seems probable therefore that the incidence of haemophilia (haemophilia A) compared with that of Christmas disease (haemophilia B) is about 5.5:1. The incidence in small series naturally varies from centre to centre. Koller (1954a) in Switzerland recorded nine cases of Christmas disease to ten cases of haemophilia. Fantl & Sawers (1954) in Australia reported a ratio of 6:30. Our own ratio (in London) is 5:20. Biggs & Macfarlane (personal communication, 1954) at Oxford, however, found only six cases of Christmas disease to 73 cases of haemophilia.

PTA deficiency appears to be rarer than Christmas disease. Three examples occurring in one family were described by Rosenthal *et al.* (1953), and four examples in one family by Frick (1954). Fifteen more patients suffering from a mild form of the disease have recently been reported (R. L. Rosenthal, 1954).

[1] See Biggs, p. 5 of this number of the Bulletin.—Ed.

6. Inheritance

a. Haemophilia (Haemophilia A)

Inheritance of true haemophilia (AHG deficiency) is of a sex-linked character which follows a well-known pattern. It is transmitted to males through healthy females, who are themselves carriers of the gene in an X sex chromosome. The carrier females do not as a rule suffer from any symptoms, and individual patients cannot be distinguished with certainty from healthy females by means of any laboratory test so far devised. However, when considered as a whole group, female carriers do appear to be more likely to suffer from abnormal haemorrhage than healthy women—for instance, after tooth extraction (Merskey & Macfarlane, 1951). Occasionally, too, laboratory tests may give results just outside the normal range. A single gene for haemophilia is thus completely or almost completely recessive in the female. It is theoretically possible for a female to have overt haemophilia if a gene is inherited from both mother and father, and an occasional example of this has been described (Merskey, 1951; Israëls, Lempert & Gilbertson, 1951). Brinkhous & Graham (1950) have also succeeded in producing haemophilia in female dogs by in-breeding.

b. Christmas Disease (Haemophilia B)

Biggs *et al.* (1952) were the first to show that Christmas disease is inherited in a sex-linked manner in the same way as haemophilia. This has been confirmed by many workers. For example, Koller (1954a) showed that four members of the von Tenna family, described in the monograph by Bulloch & Fildes (1911) as showing the classical inheritance pattern of haemophilia, were in reality suffering from Christmas disease. There may, however, be a difference between the expression of the genes of haemophilia and Christmas disease, for, in the latter, mildly affected females (giving definitely abnormal results in laboratory tests) seem to be more frequently encountered. For instance, the mother of the subject described as Case 4 by Biggs *et al.* (1952) had a lengthened clotting time and deficient prothrombin consumption, and Biggs & Macfarlane (personal communication, 1954) have recently demonstrated a deficiency of the Christmas factor in another female patient. A similar instance was reported by Brinkhous, Langdell, Penick, Graham & Wagner (1954). The remaining 68 patients referred to in the literature have been males.

c. Plasma Thromboplastin Antecedent (PTA) Deficiency

The examples of this condition so far reported suggest that the defect is transmitted to either sex by an autosomal dominant gene. Of 18 patients reported by R. L. Rosenthal (1954), ten were females and eight males.

7. Diagnosis

The first step in diagnosis is the separation of the thromboplastin-precursor deficiency states from other causes of haemorrhagic disease; the second stage of the investigation is the separation of the three disorders one from the other.

It is not possible to distinguish between the three disorders on the basis of symptoms alone. Although the haemorrhagic manifestations have been mild in most of the patients so far reported as suffering from PTA deficiency, clinically mild examples of haemophilia and Christmas disease also occur. It is always worth while to inquire into the family history.

As already mentioned, the inheritance of haemophilia and Christmas disease is similar; that of PTA deficiency appears to be distinct. However, a positive family history cannot always be obtained in either haemophilia or Christmas disease. For instance, Merskey (1950) failed to find evidence of inheritance in 28 of 72 " haemophiliacs " (39%) examined by him.

From the laboratory point of view, the separation of the thromboplastin-precursor deficiency states from the other forms of haemorrhagic disorder is comparatively easy. In the former group, the platelet count, fibrinogen content of the plasma, and prothrombin time are normal, and the presence of anticoagulants in the plasma cannot usually be demonstrated. The most important positive findings are a prolongation of the whole-blood coagulation time, with impairment of thrombin generation and defective utilization of prothrombin. However, a normal whole-blood coagulation time does not exclude a coagulation defect. In Merskey's series, 17 of 51 patients with haemophilia had a coagulation time of less than 12 minutes (Merskey, 1950). The clotting times of 15 patients with PTA deficiency were reported to be normal by R. L. Rosenthal (1954).

Further differentiation between haemophilia, Christmas disease and PTA deficiency may be made by means of mixing tests, or more specifically by the thromboplastin-generation test. Mixing tests depend upon the principle that bloods deficient in the same constituent will not have their deficiency corrected by mixing, whilst bloods deficient in different factors will mutually correct each other's defect. For example, the coagulation time of haemophilic blood is shortened by the addition of small amounts of normal blood or by the addition of Christmas-factor-deficient blood, both of which contain AHG, but not by the addition of haemophilic blood also lacking AHG. Some typical results of mixing tests are shown in Table II.

TABLE II. COAGULATION TIMES OF PLASMA OF PATIENTS WITH CHRISTMAS DISEASE AND HAEMOPHILIA, AFTER THE ADDITION OF VARIOUS TEST SUBSTANCES

Substance added (10 % concentration)	Coagulation times after recalcification (sec.)	
	Christmas-disease plasma	Haemophilic plasma
Saline	230	495
Normal plasma	105	120
Fibrinogen fraction	235	170
Haemophilic plasma	110	495
Christmas-disease plasma	230	180
Normal serum	70	360

Differentiation is best made by the thromboplastin-generation test, for the separation of haemophilia from Christmas disease can be made even if control samples obtained from proven cases are not available for study. In Table III are shown the results obtained with this test using the plasma and serum of a patient with haemophilia, and in Table IV are shown the results of a similar study made on a patient with Christmas disease. In Table V are illustrated the characteristic reactions in the three diseases. It is also possible to assay by a modified method the degree of AHG deficiency (Biggs, Eveling & Richards, 1955).

TABLE III. RESULTS OF A THROMBOPLASTIN-GENERATION TEST ON A PATIENT WITH HAEMOPHILIA

(The reacting mixture was tested at one-minute intervals for its thromboplastin activity by adding sub-samples to high-spun plasma. The figures refer to the clotting times of this plasma in seconds.)

Times of sub-sampling (min.)	Reacting mixtures		
	Normal $Al(OH)_3$-absorbed plasma, normal serum, normal platelets	Patient's $Al(OH)_3$-absorbed plasma, normal serum, normal platelets	Normal $Al(OH)_3$-absorbed plasma, patient's serum, normal platelets
1	65	67	37
2	14	37	11
3	11	22	12
4	12	23	11.5
5	11.5	22	12
6	13.5	22	11.5

The thromboplastin-generation test is the most sensitive of the available tests. It is particularly valuable in the diagnosis of mild cases of haemophilia or Christmas disease, in which the coagulation time is normal and the prothrombin-consumption test nearly so. In PTA deficiency, the defect in thromboplastin generation can be corrected by either normal serum or normal plasma; defective thromboplastin generation can be demonstrated only if both the plasma and serum of the patient are used in the test (Biggs & Macfarlane, personal communication, 1954).

8. Treatment

The aim in the treatment of serious haemorrhage is to correct as far as possible the defect in the coagulation mechanism. For this purpose, fresh whole blood or fresh plasma is usually administered intravenously. Whole blood contains AHG, Christmas factor and PTA; it is thus a potentially effective form of temporary replacement therapy for a deficiency of any of these factors.

a. Treatment of Haemorrhage in Haemophilia

Without laboratory facilities it is impossible to determine how much whole blood or plasma should be given to combat

TABLE IV. RESULTS OF A THROMBOPLASTIN-GENERATION TEST ON A PATIENT WITH CHRISTMAS DISEASE

(The reacting mixture was tested at one-minute intervals for its thromboplastin activity by adding sub-samples to high-spun plasma. The figures refer to the clotting times of this plasma in seconds.)

Times of sub-sampling (min.)	Reacting mixtures		
	Normal $Al(OH)_3$-absorbed plasma, normal serum, normal platelets	Patient's $Al(OH)_3$-absorbed plasma, normal serum, normal platelets	Normal $Al(OH)_3$-absorbed plasma, patient's serum, normal platelets
1	45	60	60
2	23	35	43
3	11.5	11.5	43
4	13	12	44
5	11	11.5	47
6	—	13	49

TABLE V. THE THROMBOPLASTIN-GENERATION TEST USING PLASMA AND SERUM DEFICIENT IN ANTIHAEMOPHILIC GLOBULIN (AHG), CHRISTMAS FACTOR OR PLASMA THROMBOPLASTIN ANTECEDENT (PTA)

$Al(OH)_3$-absorbed plasma	Serum	Generation of thromboplastin
Normal	Normal	Normal
Haemophilic	Normal	Poor
Normal	Haemophilic	Normal
Christmas disease	Normal	Normal
Normal	Christmas disease	Poor
PTA deficient	Normal	Normal
Normal	PTA deficient	Normal
PTA deficient	PTA deficient	Poor

serious haemorrhage in haemophilia. Even with expert laboratory control this is a difficult problem. The whole-blood coagulation time is not a good index to use as a guide, for the coagulation time can be easily reduced to normal, and yet the patient may continue to bleed. This is because blood may generate sufficient thrombin to clot within the normal time even though the mechanism of thromboplastin formation is still grossly inefficient. The prothrombin-consumption index is a better guide, but, even when this test gives normal results because of transfusion, haemorrhage may continue.

It can be shown by means of the thrombin-generation test (Pitney & Dacie, 1953) that large volumes of plasma have to be transfused to correct the coagulation defect in haemophilia. By a modification of the thromboplastin-generation test it has been shown that if the AHG content of the patient's plasma is kept above 30%, further bleeding is unlikely (Macfarlane *et al.* 1954). AHG is unstable in stored plasma, and results with the thrombin-generation test indicate that about 50% of the potency of plasma may be lost during storage for 10 days at 4° C. For this reason, if plasma is decided upon as the treatment of choice for a haemophiliac, it is important to use fresh plasma, preferably not more than 24 hours old. Heparinized plasma has been claimed to be superior to citrated plasma (van Creveld & Paulssen, 1952); this interesting observation needs further study.

The antihaemophilic factor in plasma is contained in the fibrinogen fraction (Cohn's fraction I). Such fractions, prepared from normal plasma, retain their antihaemophilic potency for long periods of time when freeze-dried. They are useful when fresh plasma is not available. As they can be reconstituted into a volume smaller than that of the plasma from which they were prepared, they may be injected by syringe. However, the same degree of laboratory control must be employed as when fresh plasma is used. An effort should be made to increase the AHG content of the blood to 30% or more, by the injection of relatively large volumes of the concentrate. It is important to realize that the fibrinogen fraction contains AHG and not the Christmas factor. It is therefore useless in the treatment of Christmas disease. Hence, it is essential that the defect be accurately diagnosed before therapy is begun.

Macfarlane *et al.* (1954) have recently prepared AHG from bovine plasma. Bovine blood has about 16 times the AHG potency of human blood, as estimated by the thromboplastin-generation test. A preparation of bovine AHG has been produced of which 100 mg. have an activity equivalent to two pints[1] of normal human blood. Preliminary trials of this preparation when given intravenously showed it to be effective in increasing the AHG content of the blood of haemophiliacs to such an extent that dental extractions could be performed without incident.

b. Treatment of Haemorrhage in Christmas Disease

The treatment of haemorrhage appears to be less difficult in Christmas disease than in haemophilia. This may be because the deficiency of Christmas factor is seldom so great as that of AHG, or because less Christmas factor is required to correct a coagulation defect. Normal plasma is relatively richer in Christmas-factor activity than in AHG activity (Beaumont, Caen & Bernard, 1954). Haemorrhage in Christmas disease usually ceases rapidly following whole-blood or plasma transfusions. Moreover, it is not necessary to use fresh plasma. As the Christmas factor is stable on storage, and also present in serum, either plasma or serum may be used. The duration of the effect of replacement therapy is not yet known; it seems likely, however, to be longer than in haemophilia. Beaumont *et al.* (1954) claimed that Christmas factor persists for two to three weeks in the recipient's circulation after transfusion. White, Aggeler & Emery (1953) are proceeding with efforts to purify the Christmas factor, and it is probable that purified preparations will soon be available for therapy. As emphasized previously, accurate diagnosis is essential before the use of such preparations.

c. Treatment of Haemorrhage in Plasma Thromboplastin Antecedent (PTA) Deficiency

R. L. Rosenthal (1954) stated that stored plasma is effective in correcting the coagulation defect in this condition. No information is available concerning the relative effects of fresh or stored plasma or serum in the treatment of the disease.

[1] 1 pint = 0.568 l.—ED.

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CIRCULATING ANTICOAGULANTS

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1 Incidence and occurrence of circulating anticoagulants
  a Circulating anticoagulants complicating haemophilia or Christmas disease
  b Circulating anticoagulants following pregnancy
  c Circulating anticoagulants in miscellaneous cases
2 Mode of action
3 Properties
4 Diagnosis
5 Treatment
6 Course and prognosis
7 Pathogenesis
8 Conclusions
References

The circulating anticoagulants, which are the subject of this paper, prevent the formation of blood thromboplastin but have no action on formed blood thromboplastin, thrombin or prothrombin; they are found complicating haemophilia or causing an acquired haemorrhagic diathesis resembling haemophilia. This definition specifically excludes heparin or heparin-like substances which, compared with circulating anticoagulants, are an excessively rare cause of haemorrhagic disease in man. The acquired haemorrhagic diathesis caused by these circulating anticoagulants will not be given any general name in this communication. The terms pseudo-haemophilia and acquired haemophilia-like disease have been used to describe cases with circulating anticoagulants but are confusing as they have also been applied to other conditions.

Aggeler, White, Glendening, Page, Leake & Bates (1952) and Biggs, Douglas, Macfarlane, Dacie, Pitney, Merskey & O'Brien (1952) have shown that patients thought to have haemophilia may be divided into two main groups according to whether there is a deficiency of antihaemophilic globulin (AHG), or Christmas factor or plasma thromboplastin component (PTC). There is yet no general agreement as to terminology; the condition associated with AHG deficiency will therefore be designated haemophilia, and the other condition, associated with a deficiency of Christmas factor, Christmas disease.[1]

1. Incidence and Occurrence of Circulating Anticoagulants

Circulating anticoagulants have been reported in three groups of individuals: in patients thought to have haemophilia, in females following pregnancy, and in a miscellaneous group of patients in whom haemophilia, Christmas disease or a preceding pregnancy were excluded. It should be noted that when a male patient with a haemorrhagic tendency dating from early childhood develops a circulating anticoagulant it may be impossible to determine whether the pre-existing haemorrhagic state was haemophilia or Christmas disease. In this communication these patients are presumed to have haemophilia unless it has been proved otherwise.

a. Circulating Anticoagulants Complicating Haemophilia or Christmas Disease

There have been only 17 further case reports of the appearance of circulating anticoagulants in patients presumed to have haemophilia, since the first account of such a case by Lawrence & Johnson in 1941. The development of a circulating anticoagulant is thus an uncommon complication of haemophilia. Hougie & Fearnley (1954) report that, in a series of 70 cases of haemophilia (whose records were made

[1] See also Pitney & Dacie, p. 11 of this number of the Bulletin.—Ed.

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available to them), circulating anticoagulants were found in only two, although the very sensitive thromboplastin-generation test was used for their detection. Frommeyer, Epstein & Taylor (1950) recorded that, in a series of 22 patients presumed to have haemophilia, five out of 16 who had received injections of an AHG preparation (Cohn's fraction I)[2] developed circulating anticoagulants, while no anticoagulants were detected in the remaining six patients of the series who had received only transfusions with whole blood or plasma. The conditions under which these anticoagulants developed may have been exceptional, for the activity of all of them appears to have been considerably less than that of those found in the two cases referred to above and reported by Hougie and Fearnley. Soulier & Larrieu (1953), in a study of 33 cases, found circulating anticoagulants in three. In one of these patients the circulating anticoagulant had later completely disappeared, and these authors were then able to demonstrate that this patient had Christmas disease; this diagnosis was probable in another of these patients, but the presence of the circulating anticoagulant made conclusive tests impossible. Lewis & Ferguson (1953) mention that they studied a patient with a circulating anticoagulant and when this had almost disappeared they were able to demonstrate that the basic disease was Christmas disease (PTC deficiency) and not classical haemophilia (AHG deficiency).

b. Circulating Anticoagulants Following Pregnancy

There have been 12 recorded cases in which the circulating anticoagulants were detected after pregnancy (Madison & Quick, 1945; Chargaff & West, 1946; Fantl & Nance, 1946; Hewlett & Haden, 1949, two cases; Heinle, Weisberger, Vignos & Holden, 1949; Dreskin & Rosenthal, 1950; Biggs & Macfarlane, 1953; Frick, 1953; Beaumont, 1954; Evans, 1955, in press), and it is probable that the cases described by Rosenthal, Vogel & Beres (1937) and by O'Brien (1954) were of a similar nature although no circulating anticoagulants were detected. In all but one of these patients the diathesis appeared not later than 12 months after parturition. The haemorrhagic manifestations in these subjects were usually severe and there is probably a much larger group of patients in whom the haemorrhagic manifestations, because of their mildness, do not lead the patient to seek medical advice.

c. Circulating Anticoagulants in Miscellaneous Cases

Circulating anticoagulants may be associated with—and presumably cause—an acquired haemorrhagic diathesis in males, and in females in whom pregnancy can be excluded. In three of the cases the patient was previously healthy (Joules & Macfarlane, 1938; Pons & de Torregrosa, 1952; Hougie, 1953). The remaining cases had some associated illness: tuberculous lymphadenopathy (Lozner, Jolliffe & Taylor, 1940), chronic nephritis and syphilis (Conley, Rathbun, Morse & Robinson, 1948), enlarged lymph nodes due to reactive hyperplasia of unknown origin (Conley *et al.* 1948), myocardial infarction (Singer, Mond, Hyman & Levy, 1950), rheumatoid arthritis with amyloid deposits in the lymph nodes (Collins & Ferriman, 1952) and rheumatic heart disease (Hardisty, 1954). In two cases the circulating anticoagulant appeared during the treatment of pemphigus with arsenic (Quick & Stefanini, 1948; Dieter, Spooner & Pohle, 1949).

In none of these patients is there any record of blood transfusions or injections of blood or blood products, and all but one were over 55 years of age. The condition is probably commoner than the small number of reported cases indicates.

2. Mode of Action

Biggs, Douglas & Macfarlane (1953a) have shown that physiological blood coagulation is dependent on the formation of a powerful thromboplastin by five essential components acting in the presence of calcium. These components are Factor V, AHG, Factor VII, Christmas factor and platelets. AHG and Factor V are both present in normal plasma treated with aluminium hydroxide to remove prothrombin, and normal serum contains both Christmas factor and Factor VII. In a subsequent study the same workers (Biggs, Douglas & Macfarlane, 1953b) showed that pre-incubation, in the presence of calcium, of either Christmas factor and AHG, or of platelets, Factor V and Christmas factor, led to the formation of intermediate products which would form thromboplastin more rapidly when the remaining components were added than if all five were incubated simultaneously. These workers also showed that Factor VII did not take part in any appreciable time-consuming preliminary reaction. Hougie & Fearnley (1954) studied three circulating anticoagulants which were present in two haemophilic patients and in a previously healthy individual respectively. They demonstrated that if AHG, normal serum and calcium were pre-incubated and an excess of any of the three circulating anticoagulants subsequently added, the further addition of Factor V and platelets to complete the thromboplastin system led to full thromboplastin formation. On the other hand negligible amounts of thromboplastin were generated if a circulating anticoagulant was added before instead of after pre-incubation. If platelets, serum and Factor V, or AHG, Factor V and platelets, were pre-incubated in the presence of calcium, a circulating anticoagulant being added immediately before or after pre-incubation, and each system completed to contain the same final components, it was found that little thromboplastin was formed. It follows that the circulating anticoagulant inhibits a reaction occurring in the presence of calcium between AHG and serum, or to be more specific (by inference from the work of Biggs *et al.* (1953b) quoted above) between AHG and Christmas factor. Hougie and Fearnley also showed that the platelets in these three cases were normal in respect of their ability to form blood thromboplastin and that the circulating anticoagulants had no action on formed thromboplastin. The circulating anticoagulants are therefore antithromboplastinogens—according to the terminology of Quick (1953)—and not antithromboplastins.

It is known that AHG is consumed during clotting, while Christmas factor is present in serum in at least the same amounts as in plasma (Douglas & Biggs, 1953). It is therefore reasonable to assume that Christmas factor acts on AHG substrate to form an intermediate product. The circulating anticoagulant may therefore act by directly inhibiting AHG or Christmas factor, or it may prevent the reaction between these two factors without destroying either. In all three instances the final result would reveal itself as an apparent deficiency of AHG, for this factor can be assayed only by its ability to form thromboplastin when added to Christmas factor and the remaining components of the thromboplastin system; it follows that the absence of AHG activity in the presence of a circulating anticoagulant does not necessarily

[2] See Kekwick, p. 54 of this number of the Bulletin.—Ed.

indicate that the circulating anticoagulant is directly inhibiting this factor, as was previously assumed by many workers before the discovery of Christmas factor.

The distinction between these possibilities is difficult. So far, attempts to separate either the Christmas factor or AHG from plasma samples containing anticoagulants have proved unsuccessful. Some evidence can be derived from patients recovering from circulating anticoagulants. Evans (1955, in press) and Biggs & Macfarlane (personal communication, 1954) have each studied such a case; these workers each found a deficiency of AHG with a normal serum content of Christmas factor at a time when there were no demonstrable circulating anticoagulants in patients who had developed circulating anticoagulants following pregnancy. These findings are attributable to a fall in the titre of the circulating anticoagulants, as these were previously demonstrable, to a level just sufficient to neutralize part or the whole of the patients' AHG; it may therefore be concluded that the circulating anticoagulants in these cases were directed specifically against AHG. The findings of Lewis and Ferguson, and of Soulier and Larrieu, of a deficiency of Christmas factor in patients who had circulating anticoagulants might appear to conflict with the findings of Evans and of Biggs and Macfarlane; but this is not necessarily so, as the patients of the former workers probably had a basic congenital deficiency of Christmas factor, the circulating anticoagulant having been acquired later. These cases are of great interest from an aetiological point of view and will be considered later.

3. Properties

The circulating anticoagulants studied by Hougie and Fearnley were identical in all respects and resembled in all important details the circulating anticoagulants reported by Munro (1946), Biggs & Macfarlane (1953), Collins & Ferriman (1952) and Hardisty (1954). The circulating anticoagulants are relatively heat-stable, surviving heat at 65° C. for at least five minutes, and are very stable on storage; the author has been able to demonstrate the presence of a circulating anticoagulant in serum kept at 5° C. for 18 months. The circulating anticoagulants are not adsorbed by aluminium hydroxide. They are present in the γ-globulin fraction of both plasma and serum and are precipitated by a one-quarter to one-third saturation with ammonium sulphate. Circulating anticoagulants are active over a wide pH range and are non-dialysable; they cannot be extracted by ether or chloroform.

While the majority of circulating anticoagulants have conformed to the above description, differences have been found. Some of these may be due to variations in technique, while others can now be explained in the light of recent work. It was shown by Hougie and Fearnley that the recalcification time of platelet-rich plasma containing a circulating anticoagulant shortens on standing, although the titre of the circulating anticoagulant remains unchanged. It was subsequently shown by Hougie (1955) that this change, which also occurs in normal and haemophilic platelet-rich plasma, is caused by the platelets having acquired increased coagulant properties. It follows that techniques for measuring or testing anticoagulant activity that depend on the ability of relatively small amounts of the patient's plasma to prolong the clotting time of normal plasma or whole blood may give rise to erroneous results. This could account for the apparent disappearance of the circulating anticoagulants on storage as reported by Hardisty (1954), Dieter *et al.* (1949), Soulier & Burstein (1948) and others, although each of these circulating anticoagulants was relatively heat-stable.

Some workers (Fantl & Nance, 1946; Pons & de Torregrosa, 1952) have claimed that the circulating anticoagulant studied by them was an antithromboplastin. Their conclusion is based on the finding that with very dilute solutions of a brain extract the one-stage " prothrombin time " is raised above normal. This result, however, is inevitable in all conditions in which the recalcification time is raised, as in uncomplicated haemophilia; for as the dilution of brain is increased so does it approximate to saline, the prothrombin time (" brain time ") merging into the calcium clotting time. Fantl and Nance went further and claimed that the circulating anticoagulant studied by them was a species-specific antithromboplastin. This conclusion was partly based on an experiment in which varying dilutions of rabbit and human brain were used to determine the clotting times of the patient's and normal blood. These workers observed no difference between the clotting times of the patient's and normal blood when rabbit brain was used, even in high dilutions, but a significant difference with very dilute human brain extracts in concentrations lower than 0.006 %. I cannot accept their interpretation of this experiment, which has hitherto gone unchallenged: I believe that the rabbit brain extract was not sufficiently diluted since, provided the recalcification time of the patient's plasma was raised, a divergence between the prothrombin times of the patient's and normal plasma would then have resulted.

4. Diagnosis

The most sensitive and reliable test for the detection of a circulating anticoagulant is the thromboplastin-generation test of Biggs *et al.* (1953a). If a circulating anticoagulant is present, little or no thromboplastin is generated on the incubation of any of the following three pairs of reagents in the presence of platelets and calcium: patient's serum and normal $Al(OH)_3$-treated plasma, patient's $Al(OH)_3$-treated plasma and normal serum, and 50 % normal and 50 % patient's $Al(OH)_3$-treated plasma and normal serum; in this way classical haemophilia, Christmas disease and the haemophilia-like disease (PTA deficiency) recently described by Rosenthal, Dreskin & Rosenthal (1953) are respectively excluded.

The comparison of titres of the circulating anticoagulants on different days is best accomplished by storing serum samples from the patient and testing all of them together at the end of the period (the anticoagulant being stable on storage). The specimens of serum to be compared are treated with aluminium hydroxide to remove any prothrombin, Factor VII or Christmas factor. The highest dilution (titre) of each specimen of serum which, when added to a normal thromboplastin system, will inhibit thromboplastin generation is then determined.

Other methods for the detection of a circulating anticoagulant depend on the ability of small proportions of the patient's blood to prolong the clotting time of normal blood. Either whole blood (Singer *et al.* 1950) or plasma may be used. If plasma is used, both the normal and the patient's plasma must be platelet-deficient to avoid a shortening of the clotting times of the plasmas on standing, because of platelet activation. The tests should be performed within two hours of the

venepunctures, as the recalcification times of platelet-deficient plasmas lengthen on standing (Hougie, 1955).

Once a circulating anticoagulant has been detected by these techniques, it should be differentiated from heparin. This is easily performed, as heparin, unlike circulating anticoagulants, can be neutralized in vitro by toluidine blue or protamine sulphate. Details of such a test are described by Biggs (1951).

It has already been mentioned that in some patients the concentration of the circulating anticoagulant is sufficient only to neutralize or inactivate whole or part of the patient's AHG (Evans, 1955, in press; Biggs & Macfarlane, personal communication, 1954). There will then be deficiency of AHG but no demonstrable circulating anticoagulant either in the plasma or serum. The condition now becomes indistinguishable by laboratory tests from classical haemophilia. Merskey (1950), in his study of the laboratory diagnosis of haemophilia, showed that the whole-blood clotting time is not a sensitive test of a deficiency of antihaemophilic globulin, while Hougie (1955) has indicated errors that may arise in using plasma clotting times. Prothrombin-consumption tests are satisfactory, but only the thromboplastin-generation test enables the specific identification of the antihaemophilic globulin deficiency without the need of obtaining plasma from a case of classical haemophilia. It is for these reasons that the thromboplastin-generation test is recommended for the diagnosis of acquired haemophilia in preference to tests which depend on the determination of whole-blood or plasma clotting times.

Diagnostic difficulties may arise in patients seen for the first time with a circulating anticoagulant, whose haemorrhagic diathesis dates from early childhood, but who have no family history of haemophilia. There have been no reports of the presence of circulating anticoagulants in cases of familial haemophilia or Christmas disease before transfusion with blood or blood products; if therefore there is no history of such transfusions the diathesis is probably acquired.

5. Treatment

The general management of patients with anticoagulants is essentially that of haemophilia. The effect of transfusion has been studied most in the haemophilic group. Munro & Jones (1943), in a study of one of these cases, found that there was no response to the transfusion of one pint[3] of fresh blood daily. Frommeyer and colleagues reported that the refractory state could be abolished by massive blood transfusions and obtained a response with 1,500 ml. of fresh blood in one of their cases. It has recently been pointed out by Macfarlane, Biggs & Bidwell (1954) that at least five pints of fresh blood daily are necessary for a patient with uncomplicated haemophilia to achieve a satisfactory haemostatic effect, and it follows that at least this amount is necessary in patients who have developed circulating anticoagulants. One of the two haemophilic patients with a circulating anticoagulant previously referred to by Hougie and Fearnley has since failed to respond to nine pints of fresh blood and two-and-a-half pints of fresh plasma given during a period of 48 hours.

Since an immunity mechanism may be concerned in the development of circulating anticoagulants, both adrenocorticotrophic hormone (ACTH) and cortisone have been used in their treatment. The reports, however, are discouraging. Hougie and Fearnley tried ACTH or cortisone in two patients, but found that the hormones did not prevent haemorrhagic manifestations or alter the titre of the circulating anticoagulants. This supports the finding of Collins and Ferriman, of Singer and his colleagues, and of Frick, but conflicts with the results of van Creveld, Hoorweg & Paulssen (1953). The latter authors, however, assessed the effect of ACTH and cortisone by determining the effect of the patient's plasma on normal whole blood—a technique which may give unreliable results. In the assessment of the results of such treatment, spontaneous fluctuations in the titres of the circulating anticoagulants must be considered.

[3] 1 pint = 0.568 l.—Ed.

6. Course and Prognosis

There is no evidence that the presence of a circulating anticoagulant in a haemophilic patient results in an increase in the number and severity of the haemorrhagic episodes; but the prognosis is adversely affected as the haemorrhagic episodes may become more difficult to control. Thus, one of the two haemophilic patients studied by Hougie and Fearnley and mentioned in the last section suffered only from a few minor bruises for a period of 14 months despite a consistently high titre of circulating anticoagulant; he then succumbed to an intraperitoneal haemorrhage which was not controlled by the transfusion of large volumes of fresh blood. Spontaneous fluctuations in the titre of the circulating anticoagulants occur, and in one of the reported cases (Soulier & Larrieu, 1953) the circulating anticoagulant completely disappeared, while in two others (Soulier & Larrieu, 1953; Lewis & Ferguson, 1953) the circulating anticoagulants had almost disappeared at the time of the reports.

The anticoagulants which developed following pregnancy have usually persisted for long periods of time. The reports of follow-up periods in the 12 recorded cases have ranged from six months to 13 years. Complete clinical and haematological recovery was observed in only three of these patients after 13 years (patient of Rosenthal *et al.* 1937, mentioned by Dreskin & Rosenthal, 1950), three years (Frick, 1953), and four months (O'Brien, 1954). Two patients improved clinically after two years and a second normal pregnancy occurred in one of these during this period. There was one fatality which occurred from asphyxia caused by a haemorrhage into the tongue. The haemorrhagic manifestations in all the reported cases have been severe. The prognosis must therefore be a guarded one.

The " miscellaneous " type of acquired haemophilia due to the formation of anticoagulants affects as a rule very elderly individuals. As recovery from haemorrhagic episodes at this time of life is very slow, the prognosis is worse than in the other two groups of cases in which circulating anticoagulants are found.

7. Pathogenesis

It is usually believed that the circulating anticoagulants found in haemophilic blood are antibodies, the antigen being the AHG present in the transfused blood. This hypothesis explains the presence of the circulating anticoagulants in the γ-globulin fraction of blood, the appearance of the circulating anticoagulants after transfusion with blood or blood products, and the finding of positive precipitin tests in some of the recorded cases. It would also explain the refractory or resistant phase which has been reported to have followed

repeated blood transfusions, the patient previously responding well to transfusions but later failing to show any clinical improvement (Munro & Jones, 1943; Pohle & Taylor, 1938). Some of these findings, however, do not bear critical analysis. In the majority of recorded cases it was not proved that the circulating anticoagulant had not been present before the patients had been given transfusions. Moreover, the response of haemophiliacs to transfusion has not always been satisfactory even in the absence of a circulating anticoagulant. This failure to respond is the expected theoretical result, for the amount given in such cases has rarely approached the five pints daily of fresh blood or the plasma from this volume necessary to achieve temporary haemostatic control (Macfarlane *et al.* 1954). The beneficial results of the smaller amounts of blood usually given in haemophilia may therefore be due to factors other than AHG against which a circulating anticoagulant, if present, might have little effect.

The circulating anticoagulants that occur following pregnancy are generally attributed to some maternal iso-immunization process. Most of the circulating anticoagulants have appeared within a few weeks or months of pregnancy and so support the hypothesis, but periods of 12 and 13 months have elapsed in two cases before the appearance of the circulating anticoagulant. An immunization process has also been invoked to explain the development of the circulating anticoagulants in the third or miscellaneous group of cases, on the grounds of their common association with diseases such as syphilis or rheumatoid arthritis, but the type or mechanism of the immunization process has never been specified.

The immunization theories have certain disadvantages. Many of the patients had no initial AHG deficiency and in these cases antihaemophilic globulin can scarcely be considered to be a foreign protein. Two of the cases (Soulier & Larrieu, 1953; Lewis & Ferguson, 1953) and possibly a third (Soulier & Larrieu, 1953) were later found to have Christmas disease. It would be interesting to know whether in these patients the anticoagulant was identical in activity with anticoagulants found in patients in whom classical haemophilia is the primary defect. Moreover, were the anticoagulant due to repeated transfusion it would be expected that the anticoagulant would occasionally arise following multiple transfusions in patients who had no primary haemorrhagic diathesis. No such cases are recorded. Another serious objection to the immunological hypothesis is the apparent identity of the circulating anticoagulants in the three groups of cases.

8. Conclusions

Circulating anticoagulants which interfere with the early stages of thromboplastin formation have been described in three types of patients: (i) in patients with a primary haemorrhagic disorder, either haemophilia or Christmas disease; (ii) in patients after pregnancy; and (iii) in a group of elderly patients, many of whom have some other disease. It appears that the development of circulating anticoagulants may be more common than was hitherto supposed. So far as can be determined the anticoagulants in the three groups have identical properties and actions.

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ROLE OF PLATELETS IN COAGULATION, THROMBOSIS AND HAEMOSTASIS, WITH SOME OBSERVATIONS ON PLATELET DYSFUNCTION, INCLUDING THROMBASTHENIA

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1 Platelets in blood coagulation
  a Formation of plasma thromboplastin
  b Coagulation factors in platelets
  c Clot retraction
2 Platelets in thrombosis
3 Platelets in haemostasis
4 Disorders associated with abnormalities of the platelets, including thrombasthenia
References

In recent years there has been a remarkable increase in our knowledge of the blood platelets and it is difficult to realize that the possibility that they might be artifacts precipitated from plasma when blood is shed was being discussed as recently as 1920 (Pianese, 1920). It is the purpose of this article to summarize some of the more important advances in our understanding of their functions, and of the results of their dysfunction.

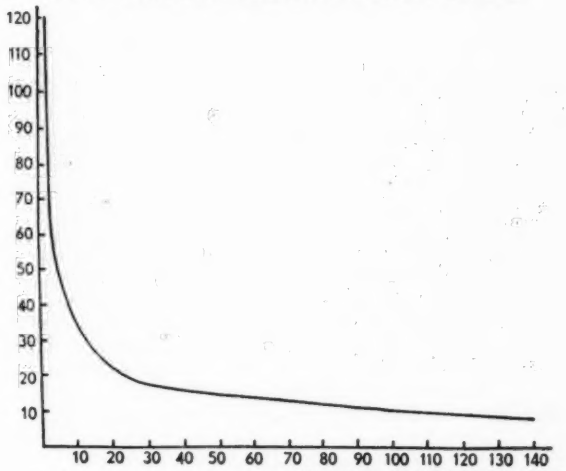
1. Platelets in Blood Coagulation

a. Formation of Plasma Thromboplastin

Platelets are essential for the coagulation of human blood. If blood is taken from a vein into a silicone-lined syringe and is transferred to a silicone-lined tube, and the platelets are then removed by centrifugation, the plasma may not clot even if it is placed in an untreated glass container (Patton, Ware & Seegers, 1948). This observation suggests that platelet disintegration on a suitable surface is probably necessary before blood coagulation can occur. Platelet disruption is probably dependent to some extent upon the action of antihaemophilic globulin, for disruption is delayed during the coagulation of plasma from haemophilic patients (Howell & Cekada, 1926), although the platelets of such patients disrupt normally during coagulation if suspended in normal plasma (Patek & Stetson, 1936).

Since Bordet & Delange (1912) first showed that platelets have thromboplastic activity, the platelets have been regarded as the source of thromboplastin when blood clots in the absence of contamination with tissue juices. Platelets, however, contain relatively little thromboplastin (Ware, Fahey & Seegers, 1948). The addition of platelets to recalcified oxalated plasma does not reduce its clotting time to less than 60–80 seconds (Biggs & Macfarlane, 1953), whereas with brain thromboplastin the clotting time can be reduced to as little as 12 seconds. It has, however, recently been shown by Macfarlane and his colleagues that an extremely potent thromboplastin is produced in plasma during coagulation. This can be demonstrated by the thromboplastin-generation test of Biggs & Douglas (1953). In this test prothrombin is removed from plasma by adsorption on to aluminium hydroxide which also removes Factor VII (Koller, Loeliger & Duckert, 1951), the Christmas factor (plasma thromboplastin component) (Biggs, Douglas, Macfarlane, Dacie, Pitney, Merskey & O'Brien, 1952) and possibly also the recently postulated Factor X of Koller (1953). The deficiencies in the factors other than prothrombin are made good by the addition of serum freed of both prothrombin and thrombin, so that, in effect, a prothrombin-free "plasma" is produced. Platelets and calcium chloride are then added and the mixture is incubated at 37° C. The thromboplastic activity that develops in the mixture is estimated at intervals by observing the coagulation times of samples of normal citrated plasma to which calcium chloride and aliquots of the above-mentioned mixture are added. As this mixture contains virtually no prothrombin, the coagulation of the normal plasma will be due almost entirely to the thromboplastin developed in the mixture, and the coagulation time will be inversely proportional to the concentration of thromboplastin. By observation of the coagulation times with different dilutions of this mixture, a thromboplastin dilution curve can be constructed relating clotting time to thromboplastin concentration (fig. 1). Figure 2 shows the thromboplastin concentrations generated in 40 different tests. The thromboplastin formed gradually over a period of 4–5 minutes and, when it had fully developed, the mean clotting time of normal plasma to which the mixture was added was less than

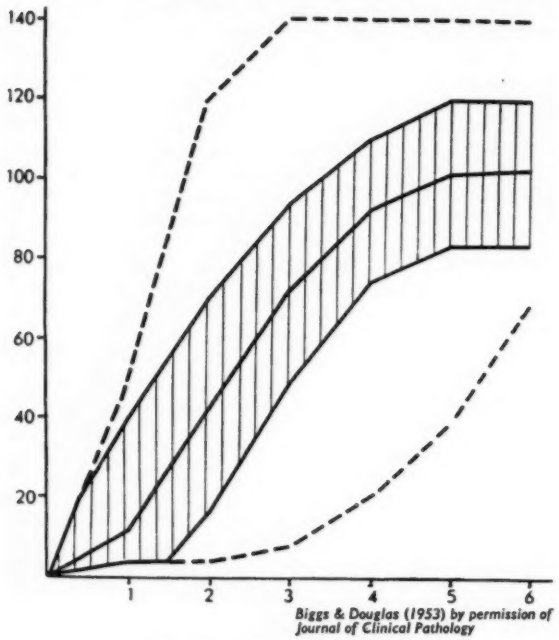
FIG. 1. THROMBOPLASTIN DILUTION CURVE



Abscissae: thromboplastin (%)
Ordinates: clotting time (sec.)

* The author is indebted to the Medical Research Council for a grant for expenses.

FIG. 2. NORMAL RANGE OF THE THROMBOPLASTIN-GENERATION TEST



Abscissae: incubation time (min.)
Ordinates: thromboplastin (%)

Central curve represents average of 40 different tests.

Shaded area shows range of variation obtained in 38 of 40 observations in which the adsorbed plasma was obtained from different normal subjects, but serum and platelets were obtained from one subject.

Extreme limits show the range of variation when all the reagents were derived from different sources.

10 seconds. This indicates a thromboplastic activity as great as, if not greater than, that of the most potent tissue extracts. Biggs, Douglas & Macfarlane (1953a, 1953b), using different combinations of factors, have shown that platelets, antihaemophilic globulin, Christmas factor, Factor V and Factor VII are all required, and that if any one of these is missing, thromboplastin formation will be impaired.[1] The reagents used by these workers probably contained the recently postulated factors X (Koller, 1953) and plasma thromboplastin antecedent (Rosenthal, Dreskin & Rosenthal, 1953); and it may well be that these factors are also necessary for normal thromboplastin generation.

The importance of platelets in the generation of plasma thromboplastin can readily be seen from the data provided in fig. 3, which shows the formation of thromboplastin in mixtures of serum and $Al(OH)_3$-adsorbed plasma containing decreasing concentrations of platelets (Biggs *et al.* 1953a).

b. Coagulation Factors in Platelets

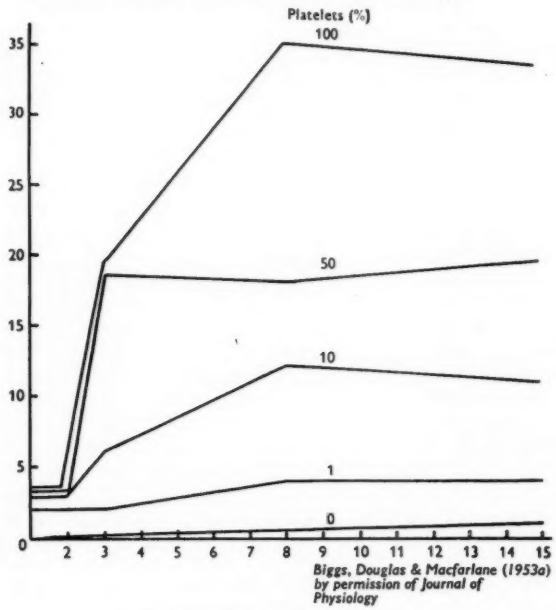
The thromboplastin-generation test has shown that platelets are necessary for the formation of plasma thromboplastin. The extraction of four coagulation factors from platelets has been reported:

Platelet Factor I. This was first described by Ware *et al.* (1948). They found it, together with platelet Factor II, in watery extracts of platelets. Platelet Factor I has an activity resembling that of Factor V of normal plasma; but unlike Factor V it is particulate, and 70–80% of this activity can be removed by centrifuging the extract at about 32,000 *g*. The Factor II activity is not removed by centrifugation (Ware *et al.* 1948). van Creveld & Paulssen (1951) have described a chemical method of separating these two factors.

Platelet Factor II. This accelerates the conversion of fibrinogen to fibrin by thrombin (Ware *et al.* 1948).

Platelet Factor III. van Creveld & Paulssen (1952) have shown that the water-insoluble fraction of human platelets (remaining after removal of Factors I and II) antagonizes the action of heparin and has thromboplastic activity. Deutsch (1954) has recently reported that these two activities are due to the presence of two coagulation factors: Factors III and IV. Factor III can convert prothrombin to thrombin in the presence of antihaemophilic globulin. Stefanini & Campbell (1954) have described a method for the purification of this factor and have shown that, in vitro, it corrects the deficient prothrombin consumption of the blood of patients with thrombocytopenia and thrombasthenia and that injection, in vivo, corrects deficient prothrombin consumption in patients with thrombocytopenia.

FIG. 3. THE FORMATION OF PLASMA THROMBOPLASTIN IN MIXTURES CONTAINING DECREASING PLATELET CONCENTRATIONS



Abscissae; time (min.)
Ordinates: thromboplastin (%)

A suspension of platelets containing about 500,000 platelets/mm.^{3} was diluted 1 in 2, 1 in 10 and 1 in 100.

[1] See Biggs, p. 5 of this number of the Bulletin.—ED.

TABLE I. EFFECT OF FREEZING PLATELETS AT −183° C. FOR 15 MINUTES, OR OF STORING THEM AT 4° C. FOR 48 HOURS, ON THEIR CAPACITY TO PROMOTE CLOT RETRACTION IN NORMAL PLASMA

(It should be noted that all the samples of plasma and platelets were obtained from the same donor.)

Date	Number of experiments	Sodium citrate (3.2% w/v) (ml.)	Platelet-"free" citrated plasma (0.4 ml. 3.2% (w/v) sodium citrate to 2.0 ml. blood), freshly prepared on day of experiment (ml.)	Platelet suspension in 3.2% (w/v) sodium citrate, freshly prepared on day of experiment (ml.)	Platelet suspension A stored at 4° C. for 48 hr. (ml.)	Platelet suspension B frozen in liquid oxygen (temperature = −183° C.) for 15 min. and then allowed to thaw at room temperature (ml.)	Thrombin in saline (40 units/ml.) (ml.)	Platelet count on platelet-"free" plasma (per mm.³)	Final concentration of platelets before addition of thrombin (per mm.³)	Clot retraction (%)
26 March 1946	4	0.4	2.0	—	—	—	0.1	200	—	Too slight to estimate
	4	—	2.0	0.4*	—	—	0.1	200	210,000	94
28 March 1946	4	0.4	1.5	—	—	—	0.1	200	—	Too slight to estimate
	3	—	1.5	0.4†	—	—	0.1	200	310,000	92
	4	—	1.5	—	0.4	—	0.1	200	187,000‡	Too slight to estimate
	2	—	1.5	—	—	0.4	0.1	200	310,000	Too slight to estimate

* An equal volume of this preparation was used to make platelet suspension A.
† An equal volume of this preparation was used to make platelet suspension B.
‡ The platelet count on the platelet suspension in citrate had fallen during storage from 1,260,000/mm.³ to 890,000/mm.³.

Platelet Factor IV. This antagonizes the action of heparin (Deutsch, 1954).

It is not known which of these factors is utilized in the generation of plasma thromboplastin, although the properties of Factors I and III suggest that they, at least, must be required.

c. Clot Retraction

After coagulation, the clot formed from normal blood contracts, extruding serum and a small number of red cells. This process can easily be measured by observing the volume of the extravasated serum and red cells, and expressing this as a percentage of the original volume of blood (Macfarlane, 1939; Ackroyd, 1949a; Budtz-Olsen, 1951).

Clot retraction depends on the presence in the blood of an adequate number of normal platelets (Arthus & Chapiro, 1908; Le Sourd & Pagniez, 1911; Tocantins, 1934). The mechanism by which platelets cause clot retraction has been the subject of much speculation. It has been claimed (Fonio, 1951, 1952a, 1952b) that clot retraction results from the activity of a substance, retractozyme, which acts on fibrin in some way to cause its shortening. Fonio states that ultrasonic irradiation of platelets enables the hyalomere to be separated from the rest of the platelet, and that this separated material contains the substance responsible for clot retraction. Rovatti (1950) on the other hand has observed that ultrasonic irradiation of platelet-containing plasma reduces its clot retraction. It seems possible that Fonio's results were due to the presence of undisrupted platelets in his preparation of hyalomeres, for there is a considerable amount of evidence that the action of platelets is a vital one. Storage at 4° C. for 48 hours, or freezing in liquid oxygen for 15 minutes, abolishes the capacity of platelets to promote normal clot retraction (see Table I). Similarly, lysis of platelets by antiplatelet serum (Tocantins, 1934) or by the lytic substance in the sera of sedormid-sensitive patients (Ackroyd, 1949b, 1949c) also inhibits clot retraction.

It has for long been believed that the fibrin strands themselves contract after coagulation, and Bailey, Astbury & Rudall (1943) have produced evidence, based on *x*-ray diffraction patterns, that this may be so. They consider that fibrinogen and fibrin are members of the keratin–myosin group of fibrous proteins, and have shown that there is an unstretched α form and a stretched β form of both fibrinogen and fibrin. Duke (1912) suggested that platelets act by anchoring the fibrin filaments together. He appears to have considered that, if the platelets were not present, the contracting fibrin strands would slip over each other and so would produce little or no reduction in the volume of the clot as a whole. Tocantins (1934) considers that the platelets cause clot retraction by settling on the fibrin threads and later fusing into small masses, so drawing the fibrin strands together. Budtz-Olsen (1951) has extended this concept. He believes, with Tocantins, that the platelets are firmly fixed to fibrin strands and that these are drawn together by the fusion of the platelets. By digesting fibrin and other proteins with trypsin during the coagulation of platelet-rich plasma, he has been able to show that the platelets send out fine processes which join with those from other platelets, and which subsequently contract. He concludes that the contraction of these processes causes clot retraction by drawing the platelets and their attached fibrin strands together.

Perhaps the most fascinating thing about clot retraction is that its function is entirely obscure. Budtz-Olsen (1951) believes that it is a "phylogenetic relic" of no value to the organism, while some authors (e.g., Lampert, 1938; Quick, 1950) even consider it to be disadvantageous to the body because it is, according to them, a factor in the production of thrombo-embolism. The generally accepted view is that originally put forward by Fonio (1921) and subsequently supported by Quick (1942), although this latter author subsequently abandoned the idea (Quick, 1950). According to this hypothesis, the clot adheres to the edges of the injured vessel and draws them together, thus securing haemostasis by acting as a physiological ligature. The force of clot retraction is, however, extremely weak. An ingenious method for its measurement has been devised by Budtz-Olsen (1951), who has shown that the force is less than 20 mm. of serum, which

is very much less than the blood pressure even in the capillaries (Landis, 1934), and would therefore be quite inadequate to maintain haemostasis. Perhaps the most reasonable view is that of Kristenson (1932), who considered that clot retraction, by causing shrinkage of the clot, restores patency to a thrombosed vessel by drawing the clot away from part of its internal surface.

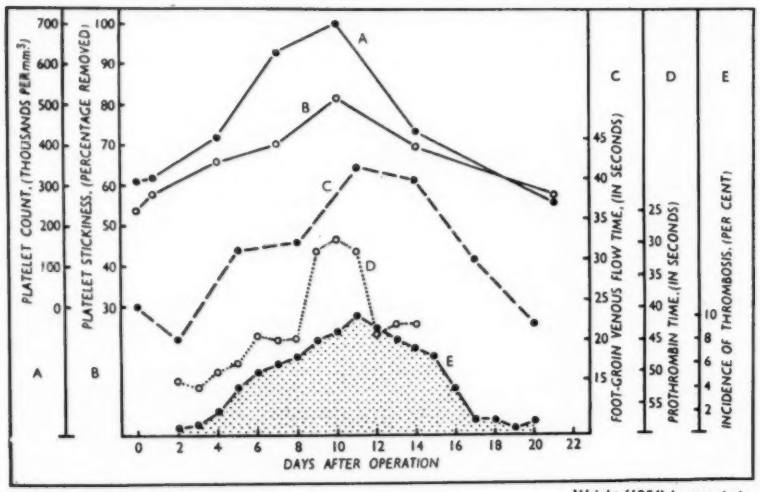
2. Platelets in Thrombosis

The importance of platelets in the initiation of thrombosis was first demonstrated by Bizzozero (1882) and Eberth & Schimmelbusch (1888) who investigated the results of injury of the intima of the mesenteric vessels of experimental animals. In those experiments in which they succeeded in inducing thrombosis, they showed that the earliest visible event was the deposition of large numbers of platelets on the injured area of the vessel wall. They also observed that slowing of the blood stream favoured thrombosis, because when blood was flowing at a normal rate the cells, including platelets, were confined mainly to the centre of the stream, being separated from the intima by a film of plasma; but, when the rate of flow was reduced, large numbers of platelets could be seen in the peripheral layer of plasma, and so were more readily able to be deposited upon injured areas of endothelium.

Ante-mortem venous thrombosis with its ever present danger of pulmonary embolism is a major hazard in the post-operative and post-partum periods, and in elderly bedridden patients. The veins most commonly affected are those of the calf muscles. From what has been stated above, it will be clear that lesions of the vascular intima, slowing of the blood stream, and changes in the blood might all predispose to thrombosis.

Very little is known of the endothelial lesions predisposing to venous thrombosis, but it has been shown conclusively that the blood flow in the veins of the legs is much reduced in bedridden patients (Smith & Allen, 1940; G. P. Wright, 1954). Also, it is well known that the number of platelets in the blood often rises considerably after surgical operations or parturition (Dawbarn, Earlam & Evans, 1928; Hueck, 1926; Øwre, 1929; Kristenson, 1929; Brock, 1933; H. P. Wright, 1942). The platelet count reaches a maximum on about the tenth day, which corresponds with the period of greatest incidence of post-operative and post-partum venous thrombosis. H. P. Wright (1942) has investigated the changes in the platelets during this period and has shown that not only do they increase in number, but they also increase in stickiness. She showed this by placing blood, kept fluid with a very small amount of heparin, in a glass tube which was slowly rotated. The platelet counts in such preparations slowly fall as a result of adhesion of platelets to the surface of the glass. Stickiness of the platelets was estimated by observing the rate of fall of the platelet count. It was found that the stickiness rose during the first 10 days after operation or parturition and then slowly returned to its normal level. The reason for this increase in stickiness is not clear. Wright considers that young platelets are more sticky than old ones and that the increased stickiness is due to the rapid outpouring of new platelets. Savitsky (1953), however, claims to have isolated a factor from plasma which he believes to be responsible for the increase in stickiness. It seems possible that the increase in platelet stickiness may merely reflect changes in other coagulation factors. Indeed, H. P. Wright (1946) herself has shown that platelet stickiness is reduced when there is a deficiency of antihaemophilic globulin. Also, G. P. Wright (1954) states that coincidentally with the rise in platelet stickiness there is a rise in the coagulability of the blood as measured by the one-stage prothrombin time of Quick (1935). Other changes occurring in the post-operative period are a slight shortening of the coagulation time (Dawbarn *et al.* 1928), and an increase in plasma fibrinogen (Macfarlane, 1954). The correlation of some of these changes with the incidence of thrombosis in the post-operative period is shown graphically in fig. 4. All these changes would suggest that a general increase in the tendency to blood coagulation is a characteristic of the post-operative and post-partum states. It must, however, be admitted that venous thromboses occur commonly in elderly, medically treated patients who are confined to bed and in whom these changes, other than the slowing of blood flow, presumably do not occur.

FIG. 4. CORRELATION OF THE INCIDENCE OF THROMBOSIS WITH OTHER CHANGES OCCURRING IN THE POST-OPERATIVE PERIOD



Wright (1954) by permission of Longmans Green

Abscissae: days after operation

Ordinates: A: platelet count (thousands/mm.³)
B: platelet stickiness (percentage removed)
C: foot–groin venous-flow-time (sec.)
D: prothrombin time (sec.)
E: incidence of thrombosis (%)

3. Platelets in Haemostasis

The spontaneous arrest of bleeding that occurs after injury to small blood vessels has been investigated by many workers. The progress of events has been observed in transparent membranes such as the mesoappendix and mesentery of the rat (M. B. Zucker, 1947), the mesentery, meninges and ear of the rabbit (Chen & Tsai, 1948) and the web of the frog's foot (Chen & Tsai, 1948); by direct microscopy of the nail-fold capillaries in man (Magnus, 1926; Macfarlane, 1941); and by serial sections of wounds in human skin (H. D. Zucker, 1949).

Damage to small arteries results in rapid vasoconstriction which, as it occurs in denervated arteries, is presumably dependent upon some local mechanism (Chen & Tsai, 1948). The vasoconstriction greatly reduces bleeding, and haemostasis is completed by the accumulation of platelets in and around the mouth of the transected vessel (M. B. Zucker, 1947; H. D. Zucker, 1949). Subsequently the extravasated blood clots firmly, and, by the time vascular spasm relaxes, the platelet plug and clot are sufficiently firm to prevent further bleeding.

The media of veins contains less muscle than that of arteries, and although some vasoconstriction does occur after injury it is much less in extent than that seen in injured arteries. Venous haemostasis depends mainly on the formation of an accumulation of platelets on the edges of the wound in the vessel wall. This accumulation increases in size until there is a large mass of platelets both within and without the vessel (Chen & Tsai, 1948; H. D. Zucker, 1949). A precisely similar chain of events occurs if an incision is made into the wall of a vein or if the vessel is cut across. In the former case, after firm clotting has occurred, the intravascular mass of platelet fragments and is washed away by the blood stream. If the platelet plug is large enough to occlude the entire lumen, the vessel becomes recanalized within a few days and the platelet plug disappears (Chen & Tsai, 1948).

Although there is considerable agreement as to the mechanism of haemostasis in small arteries and veins, there is less certainty about the control of haemorrhage from the capillaries. The main controversy is concerned with the contractility of the capillaries, and it seems probable that many of the difficulties will be resolved when it is realized that some capillaries are able to contract whilst others are not. Chen & Tsai (1948), for instance, have shown that the capillaries of the web of the frog's foot are contractile whereas those of the frog's mesentery are not, and Sanders, Ebert & Florey (1940) have produced unequivocal evidence of capillary contraction in the rabbit's ear, although capillary contraction does not apparently occur in the rabbit's mesentery (Chen & Tsai, 1948).

Macfarlane (1941), who has studied the reaction of the nail-fold capillaries in man to injury, and who supports his thesis with some beautiful photomicrographs, believes that capillary bleeding in this situation is controlled initially by capillary vasoconstriction and that this is maintained until the extravasated blood has clotted firmly, a view also held by Magnus (1924, 1926). Chen & Tsai (1948), although they have shown that capillary contraction can occur, consider that capillary haemostasis is achieved mainly by adhesion of the vessel walls at the site of injury. They state that this may happen so quickly that bleeding does not occur. M. B. Zucker (1947) has made similar observations when transecting small venules with a blunt blade. According to Chen & Tsai (1948), formed elements, particularly platelets, may become attached to the capillary walls at the site of adhesion. H. D. Zucker (1949), however, in histological studies of needle punctures of human skin, has failed to find evidence that platelet plugs play any part in capillary haemostasis. He found that the exposed lips of the capillaries were sealed by the fibrin strands in the wound, but that these strands did not enter the lumina of the transected vessels.

It has for long been known that serum, and platelet extracts, have the capacity to promote vasoconstriction (Stewart & T. F. Zucker, 1913). Recently it has been shown that this is due to the liberation of an intensely active vasoconstrictor substance from the platelets (Reid & Bick, 1942; M. B. Zucker, 1944). This substance, which has been given the name " serotonin ", is probably 5-hydroxytryptamine (Rapport, 1949). It acts only on vessels with muscular walls and not on the capillaries (Reid, 1943; M. B. Zucker, 1947). M. B. Zucker (1947) noted that when a platelet plug formed in and around an injured vessel there was vasoconstriction not only of the injured vessel itself but also of uninjured vessels near the site of bleeding. If thrombocytopenia was induced by the injection of antiplatelet serum into the animal, then platelet thrombi did not develop, and although the injured vessel contracted there was no constriction of the neighbouring uninjured vessels. Chen & Tsai (1948) have observed that if the clot is washed away from the area of injury, then the constriction of the injured vessel lasts a much shorter time than if the clot is allowed to remain. To summarize, therefore, although clot retraction, which is a function of platelets, is probably of little importance in controlling haemorrhage, yet the platelets do play a major part in securing haemostasis. They do this both by forming plugs in the orifices of injured vessels, and also by liberating a powerful vasoconstrictor which acts on the injured vessel so as to maintain its initial contraction, and also causes contraction of other vessels in the vicinity.

4. Disorders Associated with Abnormalities of the Platelets, Including Thrombasthenia

Two syndromes have in the past been attributed to functional abnormalities of the platelets: von Willebrand's disease, and thrombasthenia (Glanzmann, 1918). It is now generally accepted that von Willebrand's disease is due to an abnormality of the capillaries and that the platelets in this condition are normal. This subject has been well reviewed by Biggs & Macfarlane (1953). Since Glanzmann, in 1918, first suggested that the bleeding in some cases of non-thrombocytopenic purpura might be due to platelet dysfunction, the association of haemorrhagic disorders with abnormalities of the platelets has been increasingly recognized. Such cases are often referred to as cases of thrombasthenia although no clear-cut syndrome has, as yet, been defined. Glanzmann's original cases were familial, and many other authors have reported the familial incidence of the disease (Hess, 1916; Imerslund, 1947; Favre-Gilly, Guy, Beaudoing & Roger, 1954; Soulier & Larrieu, 1954), and for this reason the condition is sometimes called constitutional thrombopathy. The haemorrhagic tendency is often first noticed in childhood. However, abnormalities of the platelets are not confined to such cases. Thus, in the case described by Alexander & Landwehr (1949) the condition appeared in adult life and a

short time afterwards the patient developed thrombocytopenia. In the case described by Sussman, Wald & Rosenthal (1952) the haemorrhagic state also started in adult life, and in each of Hardisty's cases (Hardisty, 1953) the condition occurred as part of an extensive disorder of the bone-marrow.

Many authors have reported morphological changes in the platelets in cases of non-thrombocytopenic purpura, the most striking being the presence of a high proportion of "giant" platelets measuring up to 7 μ. or more in diameter (Hess, 1916; Hirsch, Favre-Gilly & Dameshek, 1950; Reimer & Vetter, 1952; Biggs & Douglas, 1953; Bernard, Buhot, Beaumont & Larrieu, 1954; Soulier & Larrieu, 1954). The platelets have sometimes been described as being abnormally small (Hess, 1916; Revol, 1944; André, 1952; Soulier & Larrieu, 1954). Various less striking abnormalities have also been described, particularly an unusual density of the granulomere (Glanzmann, 1918, 1952; Reimer & Vetter, 1952). The subject of platelet abnormalities in non-thrombocytopenic purpura has recently been comprehensively reviewed by Soulier & Larrieu (1954). In many instances it has been reported that the platelets showed an abnormally slight tendency to agglutinate. Frequently, clot retraction has been deficient or even absent and, in cases where it has been measured, the antiheparin activity of the blood has often been diminished. In some instances it has been reported that prothrombin consumption (Quick & Favre-Gilly, 1949) has been impaired, an abnormally large amount of prothrombin (and probably other coagulation factors) being left in the serum after clotting has occurred. Proof that this abnormality may be due to a functional defect of the platelets has been provided by Alexander & Landwehr (1949) and by Sussman *et al.* (1952), who showed that the abnormality in their cases could be corrected by the addition of normal platelets to the patient's blood or platelet-free plasma. Alexander and Landwehr showed moreover that the platelets of their patient were unable to correct the diminished prothrombin consumption of normal platelet-free plasma. More recently, several workers (Biggs & Douglas, 1953; Hardisty, 1953; Soulier & Larrieu, 1954; Bernard *et al.* 1954) have been able to show that the platelets in some cases of non-thrombocytopenic purpura are grossly deficient in their power to form plasma thromboplastin when incubated with normal serum and $Al(OH)_3$-adsorbed plasma. It is not known whether the platelets in these cases were deficient in any of the platelet factors described above. de Nicola (1954) has found the thromboplastic activity of the platelets reduced in thrombasthenia and in thrombocytopenic purpura (and, incidentally, in normal individuals during menstruation). Stefanini & Campbell (1954) have shown that the addition of purified platelet thromboplastic factor to the blood of patients with thrombasthenia corrects the impaired prothrombin consumption found in this condition. These observations would suggest that the platelets in thrombasthenia are probably deficient in platelet Factor III. van Creveld and his colleagues (van Creveld & Paulssen, 1951; van Creveld, Paulssen, Bartels & Vonk, 1953) have described two cases of non-thrombocytopenic purpura associated with an abnormally slight antiheparin activity of the blood. They attribute the condition to a partial or complete absence of the water-insoluble fraction of the platelets. In one case the antiheparin activity returned to normal for a short time after platelet transfusion (van Creveld *et al.* 1953). de Vries and his colleagues (de Vries, Shafrir, Efrati & Shamir, 1953) have observed three patients in whom the only abnormality in the blood appeared to be a failure of the platelets to promote normal clot retraction. Platelets from one of these patients failed to cause clot retraction in thrombocytopenic blood, but the addition of normal platelets to the blood of two of these patients permitted normal clot retraction. Finally, Bigelow (1953) has found the platelets deficient in serotonin activity in a number of haemorrhagic states, including thrombocytopenic and non-thrombocytopenic purpura.

As mentioned above, no clear-cut clinical or pathological syndrome has yet emerged from these investigations, although Bernard *et al.* (1954), on the basis of a detailed study of four patients, consider that they have identified two different groups of cases: one characterized by large platelets, normal clot retraction and diminished prothrombin consumption; the other by normal-sized platelets, absent clot retraction and normal prothrombin consumption. The blood of one of the two patients with a diminished prothrombin consumption was abnormally sensitive to heparin. The platelets of this patient were tested by the thromboplastin-generation test and were found to be deficient in their power to promote thromboplastin formation. The blood of the two patients with normal prothrombin consumption showed normal antiheparin activity. The platelets of one of these were tested by the thromboplastin-generation test and were shown to be able to promote normal thromboplastin formation. Cases occur, however, which will not fit into this classification. Thus, Soulier and Larrieu have described a patient (Soulier & Larrieu, 1954, Case 2, footnote, p. 88) with morphologically normal platelets which were, however, deficient in their capacity to produce thromboplastin when incubated with normal serum and $Al(OH)_3$-adsorbed plasma. Clot retraction and prothrombin consumption in this case were deficient, but the antiheparin activity of the blood was normal. Soulier & Larrieu (1954) have also reported two cases (numbers 19 and 51) with "giant" platelets and normal prothrombin consumption. The two patients described by van Creveld & Paulssen (1951) had platelets which were normal in appearance although the antiheparin activity of the blood was reduced. It is clear that further detailed investigations by modern techniques are required before the abnormalities of the platelets are fully understood or the syndromes caused by these abnormalities can be adequately classified.

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PLATELET AGGLUTININS AND LYSINS IN THE PATHOGENESIS OF THROMBOCYTOPENIC PURPURA, WITH A NOTE ON PLATELET GROUPS

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1 Idiopathic thrombocytopenic purpura
  a Cases with evidence of abnormal platelet destruction
  b Cases with no evidence of abnormal platelet destruction
  c Congenital thrombocytopenic purpura
2 Platelet groups
3 Thrombocytopenic purpura due to drug hypersensitivity
  a Thrombocytopenic purpura due to sedormid (allyl*iso*propylacetylurea)
  b Thrombocytopenic purpura due to other drugs
References

One of the earliest indications that the syndrome of thrombocytopenic purpura might be due to a factor in the blood stream that destroyed the platelets came from the experimental work of Bedson (1922), who showed that the administration of antiplatelet serum to guinea-pigs caused thrombocytopenic purpura, and that the serum not only destroyed the platelets but also damaged the capillary endothelium of these animals. These experiments are of great importance because recent work—reviewed below—has shown that thrombocytopenic purpura caused by drug hypersensitivity is almost certainly due to an antiplatelet factor in the blood of sensitized individuals, and that this factor causes lysis of platelets in the presence of the drug. Moreover, there is now evidence that some cases of idiopathic thrombocytopenic purpura may also be due to a substance in the plasma which destroys platelets.

1. Idiopathic Thrombocytopenic Purpura

a. Cases with Evidence of Abnormal Platelet Destruction

In 1925 Frank suggested that idiopathic thrombocytopenic purpura was caused by an abnormal splenic secretion, and that the favourable results of splenectomy in this disease were due to the removal of this secretion. The first claim to have demonstrated such a substance came from Troland & Lee (1938a, 1938b), who stated that splenic extracts from cases of idiopathic thrombocytopenic purpura caused thrombocytopenia when injected intravenously into rabbits. Although some workers were able to confirm this observation (Hobson & Witts, 1940; Rose & Boyer, 1941; Cronkite, 1944), the majority failed (Pohle & Meyer, 1939; Major & Weber, 1939; Hodge & Strong, 1939; Moore, 1940; Watson, 1941; Tocantins, 1939). About the same time Torrioli and his co-workers (Torrioli & Puddu, 1938) stated that splenic extracts contain a substance which inhibits the growth of megakaryocytes in tissue cultures, and that this substance is present in excess in splenic extracts from cases of idiopathic thrombocytopenic purpura.

The next advance in our knowledge came from Evans, Duane and their colleagues, who observed that some patients with acquired haemolytic anaemia had persistent neutropenia and thrombocytopenia. They therefore suggested that, since acquired haemolytic anaemia is due to a demonstrable agglutinin, the thrombocytopenia and granulopenia might have a similar cause (Evans & Duane, 1949). They later succeeded (Evans, Takahashi, Duane, Payne & Liu, 1951) in demonstrating a thrombocyte-agglutinating factor in the sera of patients with idiopathic thrombocytopenic purpura. These workers also drew attention to reports of transitory thrombocytopenia in the infants of women with idiopathic thrombocytopenic purpura: a finding which suggested that the thrombocytopenia in the infant might be due to the destruction of platelets by an abnormal factor in the mother's blood, which had passed through the placenta.

Studies of the survival of transfused platelets also appear to support the hypothesis that the platelets in idiopathic thrombocytopenic purpura are destroyed abnormally rapidly. It has been shown that, in thrombocytopenia due to bone-marrow replacement or hypoplasia, the platelets survive for about two to four or five days (Stefanini, Chatterjea, Dameshek, Zannos & Santiago, 1952a; Stefanini, Dameshek & Adelson, 1952b; Stefanini & Dameshek, 1953), but in cases of idiopathic thrombocytopenic purpura with very low platelet counts the platelets often disappear much more rapidly (in 30 minutes to 24 hours) (Stefanini *et al.* 1952a; Sprague, Harrington, Lange & Shapleigh, 1952).

Further and more convincing evidence of an antiplatelet factor in this disease came with the observation of Harrington (Harrington, Minnich, Hollingsworth & Moore, 1951) that the transfusion of blood from patients with idiopathic thrombocytopenic purpura into normal individuals often caused thrombocytopenia, occasionally with actual purpura, in the recipients (see fig. 1). He and his colleagues (Harrington, Sprague, Minnich, Moore, Ahlvin & Dubach, 1953) have now reported the results of their investigations on 35 cases of idiopathic thrombocytopenic purpura. Blood from 26 of these was transfused into normal recipients; 16 developed thrombocytopenia. Stefanini, Dameshek, Chatterjea, Adelson & Mednicoff (1953a) have reported similar findings. It is interesting to note that transfusion of blood from two of the patients with idiopathic thrombocytopenic purpura described by Harrington *et al.* (1951) caused thrombocytopenia in the recipients both before and after the donors had undergone splenectomy, although in each case the platelet count had returned to normal after the operation.

Harrington *et al.* (1953) were also able to demonstrate platelet agglutinins in the blood of 21 of 31 cases of idiopathic thrombocytopenic purpura. Four of these had autoagglutinins (i.e. agglutinins acting against the patient's own platelets). Stefanini, Plitman, Dameshek, Chatterjea & Mednicoff (1953b) found platelet agglutinins in the blood of 22 of 78 cases. In 13 of these patients an autoagglutinin was present, and in two of them it was the only demonstrable antiplatelet factor. In 12 of 15 cases splenectomy failed to cause any diminution in the titre of the agglutinin when tested with platelets from healthy individuals, although the patients'

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(FIGS. A—D)

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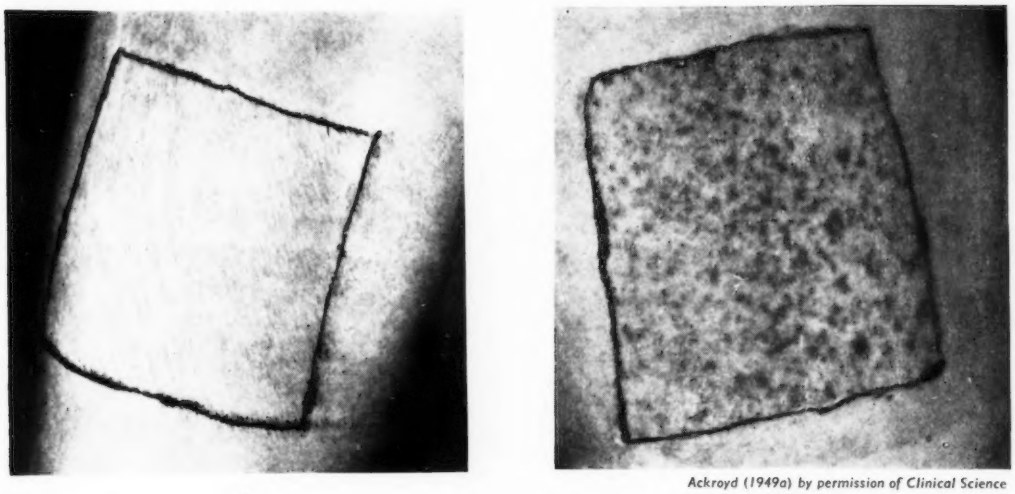

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Ackroyd (1949a) by permission of Clinical Science

FIG. A. Result of patch-testing a patient who had recovered from sedormid purpura
a: result of control test; solvent (propylene glycol) alone was used. The skin appears normal
b: result of test when sedormid in propylene glycol was used. The skin that has been in contact with sedormid is closely packed with small petechial haemorrhages

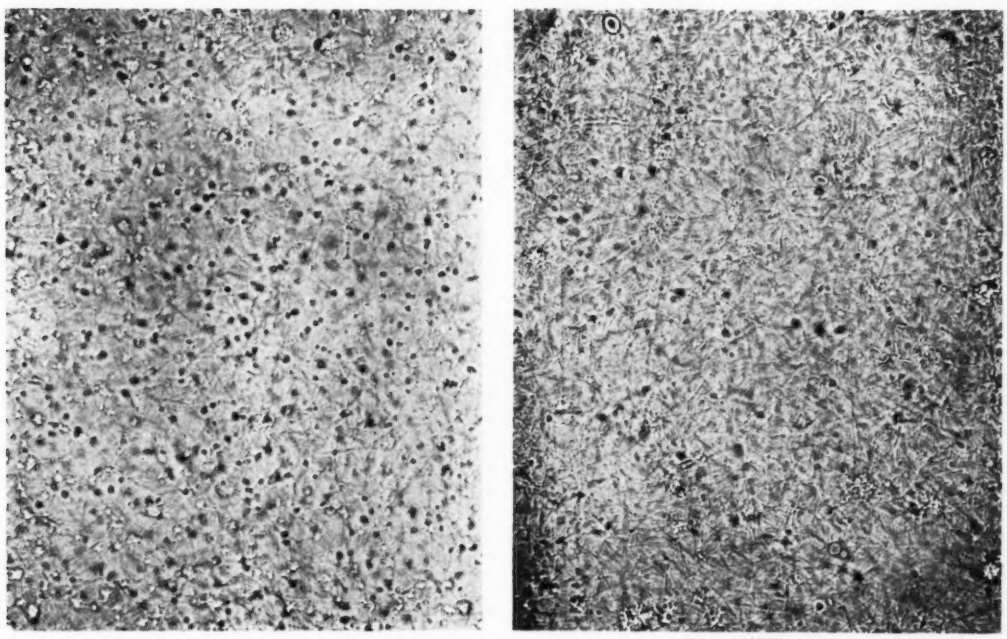

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FIG. B. Photomicrographs showing lysis of platelets by sedormid during coagulation, in a haemocytometer chamber, of platelet-rich plasma from a sedormid-sensitive patient
a: plasma + saline. The clot contains numerous platelets
b: plasma + sedormid in saline. The platelets in the clot have practically all undergone lysis

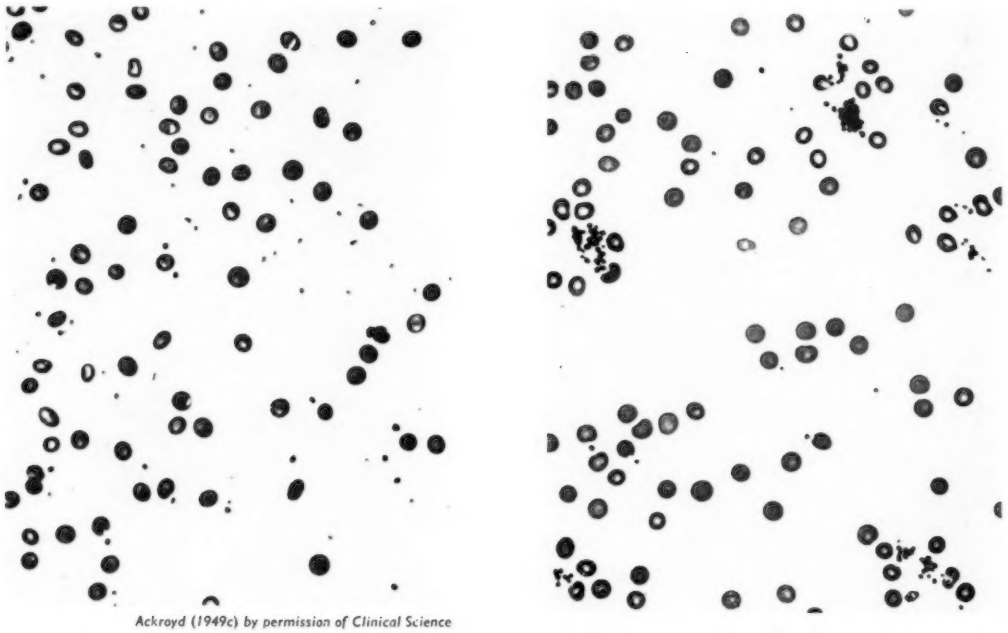

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FIG. C. Agglutination of platelets by sedormid in citrated blood from a sedormid-sensitive patient. Photomicrographs of Leishman-stained blood films taken three minutes after the blood had been mixed with isotonic sodium citrate solution or with a saturated solution of sedormid in citrate

a: blood + citrate. No platelet agglutination

b: blood + sedormid in citrate. Marked platelet agglutination

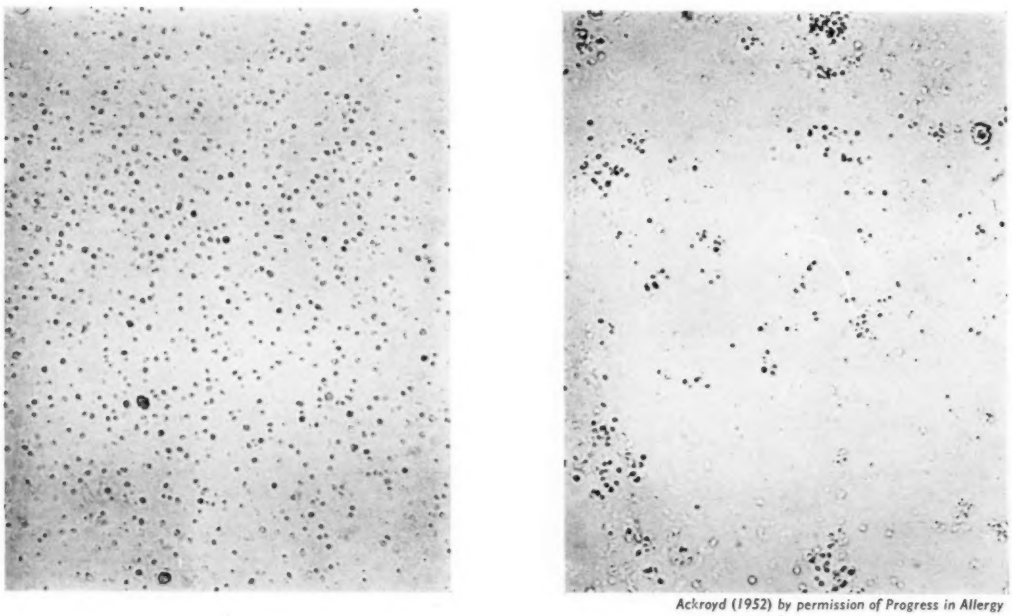

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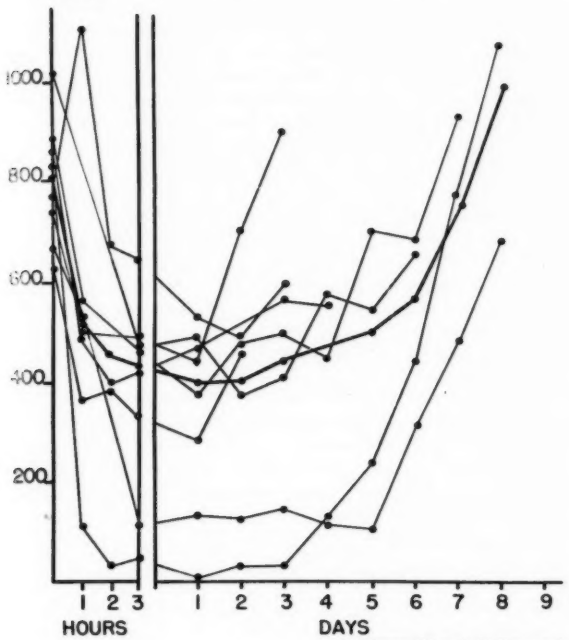
FIG. D. Platelet agglutination and lysis by sedormid in the heparinized plasma of a sedormid-sensitive patient

a: plasma + saline. No platelet agglutination or lysis

b: plasma + sedormid in saline. Considerable platelet lysis. Some platelet "ghosts" can still be seen; most of the remaining platelets are agglutinated

FIG. 1. PLATELET COUNTS OF INDIVIDUALS RECEIVING TRANSFUSIONS OF 500 ml. OF WHOLE BLOOD OR 250 ml. OF PLASMA FROM EIGHT PATIENTS WITH IDIOPATHIC THROMBOCYTOPENIC PURPURA



Harrington, Minnich, Hollingsworth & Moore (1951) by permission of Journal of Laboratory and Clinical Medicine

Abscissae: time (hours; days)

Ordinates: platelet count (thousands/mm.^{3})

The mean effect is represented by the heavy line.

platelet counts had returned to normal after the operation. In five of nine cases, however, the autoagglutinin disappeared after splenectomy, although the plasma-agglutinin titre for platelets from other sources remained unchanged (Stefanini *et al.* 1953b).

Tullis (1953) has shown that the sera of some patients with idiopathic thrombocytopenic purpura cause agglutination and lysis of normal platelets in the presence of complement. He succeeded in demonstrating this with sera from nine of 18 of his cases.

The results of the transfusion experiments, and the demonstration of platelet agglutinins and lysins, appeared to show clearly that a considerable proportion of cases of idiopathic thrombocytopenic purpura have a circulating antiplatelet factor and that this is probably the cause of the thrombocytopenia. It soon became clear, however, that the problem was more complicated, for both these groups of workers (Harrington *et al.* 1953; Stefanini *et al.* 1953b) found that they could demonstrate platelet agglutinins in the blood of some patients who had previously had transfusions or had been pregnant, but who had never suffered from purpura. Moreover, blood from two of 19 patients who had had transfusions caused thrombocytopenia when administered to normal recipients (Harrington *et al.* 1953). These findings are clearly of great importance in assessing the significance of antiplatelet factors in the blood of patients with idiopathic thrombocytopenic purpura, for most of these patients are treated by transfusions. Indeed, analysis of the cases reported by Harrington *et al.* (1953) shows that all but nine had received transfusions or had been pregnant. Of these nine, five had antiplatelet agglutinins.

Stefanini *et al.* (1953b) have also described naturally occurring platelet agglutinins in the plasma of normal individuals who have never been given a transfusion or been pregnant (see below), and it is clear that these will have to be taken into account in considering the significance of platelet agglutinins in the blood of patients with idiopathic thrombocytopenic purpura. The same authors also emphasize the technical difficulties when they state that " Negative agglutination tests would at times become positive if careful consideration was given to the optimum proportion between number of platelets in the final mixture and titer of the agglutinin ". Moreover these workers have observed two patients who had developed immune platelet agglutinins following repeated transfusions, in whom transfusion of " compatible " platelets (as judged by agglutinin tests) produced no greater rise in the platelet counts than did transfusion of " incompatible " platelets; the platelets in each case being removed from the circulation extremely rapidly. They also found that transfusion of plasma from these two patients into both " compatible " and " incompatible " recipients caused comparable and striking falls in their platelet counts. Furthermore, Harrington (1954) has observed that transfused platelets only rarely survive for a normal time in cases of idiopathic thrombocytopenic purpura, even in those cases with no demonstrable platelet agglutinins.

Another fact that makes assessment of platelet agglutinins extremely difficult is that some workers who have tried to demonstrate agglutinins in the plasma of patients with idiopathic thrombocytopenic purpura have either failed to do so or have obtained unreproducible results (Harrington, 1954; Sauer & van Loghem, 1954, and personal communications). The technique appears to be extremely difficult, and the amount of agglutination that must be considered significant is small (C. V. Moore, personal communication, 1953). So difficult is the technique that Fluckiger, Hässig & Koller (1953) have adapted the technique of Coombs, Mourant & Race (1945) for the demonstration of platelet agglutinins. This technique had previously been used by Stefanini *et al.* (1953a). Harrington (1954) and Sauer & van Loghem (1954), however, have found it unsatisfactory. In a further search for a more satisfactory method Kissmeyer-Nielsen (1953) has introduced a technique based upon the adsorption of platelet proteins on to the surface of red cells which have previously been treated with tannic acid. When suspended in a potent antiplatelet serum such cells undergo agglutination. Kissmeyer-Nielsen (personal communication, 1954) and Sauer & van Loghem (1954) have applied this method to the detection of platelet agglutinins in thrombocytopenic purpura. Sauer & van Loghem (1954) obtained fairly satisfactory results except that in three of eight cases of miscellaneous diseases, in which there was no reason to suspect the presence of antiplatelet substances, the test gave positive results. Kissmeyer-Nielsen himself obtained disappointing results with this technique. Harrington (1954) has reported that the amount of platelet agglutination produced by the sera of patients with

idiopathic thrombocytopenic purpura can be increased if the platelets are first treated with pepsin, and if calcium and magnesium ions and fresh horse serum and bovine serum are added to the patient's serum. Finally, Malinvaud & Dausset (1954) have claimed that platelet agglutination by the sera of patients with idiopathic thrombocytopenic purpura can be greatly increased if the platelet suspension is vigorously agitated for 30 minutes. It remains to be seen whether any of these newer procedures will provide a more satisfactory method for the investigation of platelet antibodies.

The significance of the thrombocytopenia caused by transfusion of blood from patients with idiopathic thrombocytopenic purpura to normal recipients is also made obscure by the finding of Stefanini & Chatterjea (1952) and of Wilson, Eisemann & Chance (1952a) that the transfusion of blood, plasma or serum from healthy individuals to normal recipients causes a striking fall in the platelet count. Stefanini & Chatterjea (1952) observed thrombocytopenia in 32 of 36 recipients in their experiments. The maximum observed fall was 76% of the original platelet count and the mean fall was over 50%. Stefanini *et al.* (1953a) found that transfusion of plasma from 14 of a series of 35 cases of idiopathic thrombocytopenic purpura caused a significant fall in the platelet count in normal recipients, but in 12 this could be distinguished only in duration from the thrombocytopenia which frequently follows the transfusion of normal plasma to normal recipients.

Finally, to make " confusion worse confounded ", Wilson, Eisemann & Chance (1952b) have made the astonishing observation that if blood or plasma from cases of idiopathic thrombocytopenic purpura is transfused to normal recipients, and their platelets are subsequently estimated, no fall in the platelet count will be observed if the platelets are counted in citrated venous blood by the method of Tocantins (1937), although a fall will be observed if they are counted in citrated capillary blood by the indirect method of Dameshek (1932) used by Harrington and Stefanini. The significance of this finding is entirely obscure.

All these observations make it extraordinarily difficult to judge to what extent idiopathic thrombocytopenic purpura is due to the presence of an antiplatelet factor in the blood stream. That such factors do sometimes cause thrombocytopenic purpura would seem to be proved by two recent case reports. The first is an extremely detailed study by Stefanini *et al.* (1953a) of a case of idiopathic thrombocytopenic purpura, with a platelet agglutinin titre of over 1 : 4,000, whose plasma, when injected into normal recipients, caused purpura and profound thrombocytopenia. The second is a case reported by Dausset, Delafontaine & Fleuriot (1952) whose serum contained an extremely powerful agglutinin which also caused platelet lysis in vitro within a few minutes. Perhaps even more convincing evidence is found in the occurrence, already mentioned, of transient thrombocytopenia in the infants of mothers who have or have had idiopathic thrombocytopenic purpura.

In conclusion, it can be said that there is strong evidence of the existence of an antiplatelet factor in the blood of some patients with idiopathic thrombocytopenic purpura. The present techniques for demonstrating platelet agglutinins are difficult and a full evaluation of the part played by antiplatelet substances in the pathogenesis of this disease must await the development of new methods for their demonstration.

b. Cases with No Evidence of Abnormal Platelet Destruction

Analysis of the cases reported by Harrington *et al.* (1953) shows that of 31 patients examined for platelet agglutinins 10 showed none, and of 26 patients whose plasma was transfused to normal individuals the plasma from 10 failed to cause any lowering of the platelet count in the recipient. Transfused platelets survived for a normal time in one patient in whom both these types of investigation gave negative results. Two patients whose plasma did not contain agglutinins were pregnant when their plasma was examined. Both were delivered of infants with normal platelet counts. Harrington *et al.* (1953) suggested that, in cases such as these, the thrombocytopenia may be due to deficient formation rather than to abnormal destruction of platelets. However, it should be noted that, in a more recent communication, Harrington (1954) has stated that only in exceptional cases of idiopathic thrombocytopenic purpura do transfused platelets survive for a normal time, even though no platelet agglutinins can be demonstrated in the plasma. It would appear, therefore, that this type of purpura is probably very uncommon.

c. Congenital Thrombocytopenic Purpura

Congenital thrombocytopenic purpura is extremely rare. Occasional cases are persistent (Landolt, 1948). Witts (1932) has described a congenital familial haemorrhagic diathesis with thrombocytopenia. Interest has recently centred around a group of cases of congenital thrombocytopenic purpura in which the disease is transitory. In most of these, the mother has had thrombocytopenic purpura (Sanford, Leslie & Crane, 1936; Davidson, 1937; Waters, 1946; Talmadge & Berman, 1947; Stroebel, Campbell & Hagedorn, 1949; Epstein, Lozner, Cobbey & Davidson, 1950; Litchfield, Sternberg & Zweifler, 1950; Robson & Walker, 1951). As mentioned above, these cases appear to be caused by the transmission of an antiplatelet factor across the placenta from the maternal to the foetal circulation. Harrington *et al.* (1953) have investigated eight infants with neonatal purpura born to three mothers each of whom had undergone splenectomy for idiopathic thrombocytopenic purpura. Two had recovered after the operation and had normal platelet counts; the third had improved only moderately. Seven of the eight children were born after the mother had undergone splenectomy. All three mothers had platelet agglutinins but in only one child could agglutinins be demonstrated. All the infants recovered rapidly.

Very occasionally, the mother has never shown either thrombocytopenia or purpura, and has never been given a transfusion. Harrington *et al.* (1953) have examined a case of transient neonatal thrombocytopenic purpura of this type. The mother had had three previous pregnancies. Her plasma contained agglutinins which acted on the infant's platelets and on platelets from other sources, but not on her own. She would seem therefore to have been immunized to platelets by pregnancy, in which case the thrombocytopenia in the infant may well have been produced by a mechanism akin to that which causes the haemolytic anaemia in erythroblastosis foetalis.

2. Platelet Groups

The evidence for the existence of platelet groups depends mainly on the observation that, if platelet-rich blood is transfused to patients with thrombocytopenia secondary to aplastic

anaemia or bone-marrow replacement, the platelets persist in the circulation for two to five days, but that with repeated transfusions the survival time of the platelets often becomes progressively shorter (Hirsch & Gardner, 1952; Stefanini *et al.* 1952b). These findings suggest that antibodies develop against the transfused platelets, and imply that there may be platelet groups which do not correspond with the red-cell groups, since in none of these reports is there any suggestion of erythrocyte incompatibility. Harrington *et al.* (1953) have investigated this possibility, using a platelet agglutination technique. The plasma samples used for typing were obtained from patients who had had repeated transfusions for refractory anaemia. On the basis of tests with 110 platelet specimens, Harrington's results indicate the existence of 12 platelet groups. Stefanini *et al.* (1953b), in a similar series of experiments, considered that they could differentiate six platelet groups, three of which were confirmed by agglutinin cross-absorption tests.

Stefanini *et al.* (1953b) have also claimed that normal plasma samples from healthy individuals, who have neither been pregnant nor have been given transfusions, contain platelet agglutinins. They cross-tested platelets and plasma from 215 individuals and, as a result of over 55,000 agglutination tests, concluded that there are two naturally occurring platelet agglutinins, from which they deduce that there are probably four platelet groups. There was no apparent relationship between any of these platelet groups and the red-cell groups (Stefanini *et al.* 1953b; Harrington, 1954). Gurevitch & Nelken (1954) and Moureau & André (1954) in their investigations, however, have found that the platelet groups correspond with the ABO red-cell groups. These observations are, of course, all based on the technique of platelet agglutination, and the contradictory results still further emphasize the need for more satisfactory methods for demonstrating platelet groups and platelet antibodies.

3. Thrombocytopenic Purpura due to Drug Hypersensitivity

Although thrombocytopenic purpura due to drug hypersensitivity is rare, it may be caused by a large variety of different compounds. Those most commonly reported are: sedormid[1], the arsenobenzol compounds[2], combined bismuth and arsenic therapy[3], the sulphonamides[4], quinine[5], quinidine[6], and gold compounds[7]. Other drugs which have been considered to have caused the condition are: digitoxin (Berger, 1952; Miescher & Ritter, 1953), iodine compounds (Dennig, 1933; Davis & Saunders, 1946), chrysarobin (Peck, Rosenthal & Erf, 1936), phenobarbitone (van Andel & Groen, 1937), allyl*iso*propyl-barbituric acid (Wintrobe, 1942), nirvanol (Jones & Jacobs, 1932), sodium salicylate (Rappoport, Nixon & Barker, 1945), insulin (Strasser, 1935), tetraethylammonium chloride (Ham, 1948), ergot (Peshkin & Miller, 1934), colloidal silver (Hertzog & Roscher, 1922), bismuth (Bianchi, 1932), streptomycin (Esser & Mueller-Jepburn, 1952; Thiele, 1952), antabuse (Leibetseder, 1952), *iso*nicotinic acid hydrazide (Baart de la Faille & Verlop, 1953), paramethadione (Reichelderfer, Pearson & Livingston, 1953), *p*-aminosalicylic acid (Le Melletier & Caulet, 1952), propylthiouracil (Fewell, Eugel & Zimmerman, 1950), ethylallylacetylurea (Graudal, 1949), Narcodorm (Amdrup, 1952), Marbadal (Kiep, 1952), amidopyrine (Kiep, 1952), and antazoline (2-(*N*-phenyl-*N*-benzylaminomethyl)-imidazoline) (Ackroyd, 1954b). Thrombocytopenic purpura has also been described as having been caused by eating mistletoe berries (Michon & Harmand, 1952), by exposure to DDT insecticide (Karpinski, 1950; Scalettar & Mazursky, 1952), or "leg stocking colour" preparations (Limarzi, 1944), by insect bites (Fatzer, 1939), and also, very rarely, by foods (Weil, 1920; Squier & Madison, 1937; Dutton, 1938; Urbach & Gottlieb, 1946). This list does not include cases where the red or white cells have been affected in addition to the platelets.

It cannot be too strongly emphasized that some other evidence, beyond the occurrence of thrombocytopenic purpura whilst the patient has been taking a drug, must be adduced before a causal relationship can be considered to be established. Proof has most frequently been provided by observing the recurrence of thrombocytopenic purpura in response to a further dose of the drug after the patient has recovered from his original attack. This should preferably be done on more than one occasion (Cooke, 1947). As this procedure is not devoid of risk, it is preferable first to try the in-vitro tests mentioned below, and to administer a further dose of the drug only if these give negative results. Negative in-vitro tests cannot be regarded as proving that the patient is not sensitive to the substance being investigated, for the tests may not be sufficiently delicate to reveal the sensitivity. This possibility is emphasized by recent reports on quinidine purpura by Bigelow & Desforges (1952) and by Barkham & Tocantins (1954). Quinidine caused platelet agglutination in the plasma of the patients described by both these groups of workers, but after a short time—in one case only 11 days after a test dose had given a positive result—platelet agglutination could no longer be demonstrated, although the patients were almost certainly still sensitive to the drug. Probably a number of different tests should always be used. Thus, in the case due to antazoline described by the author (Ackroyd, 1954b), the drug caused a marked fall in the clot retraction of the patient's blood immediately after she had recovered from an attack of thrombocytopenia induced by a test dose of the drug. Seven weeks later, however, no effect of the drug on clot retraction could be demonstrated, although it was still possible to demonstrate platelet agglutination and lysis and complement fixation by the drug. If there is any doubt as to the interpretation of the in-vitro tests, or if these are negative, the patient should be given a test dose of the drug after bleeding has ceased and the platelet count has returned to normal. The initial dose given should be very small—not more than 5 mg.—and if this produces no change in the platelet count, the dose should be cautiously increased.

[1] Dennig (1933); Loewy (1934); Falta (1936); Hadorn (1936); van Andel & Groen (1937); Lieberherr (1937); Markoff (1938); Falconer & Schumacher (1940); Moeschlin (1942); Büchler (1944); Aggeler, Howard & Lucia (1946); Ackroyd (1949a, 1949b, 1949c, 1951, 1952, 1954a); Grifoni, Volta & Vanacore (1952); Miescher & Miescher (1952); Compston & Stewart (1953); Grant (1953).

[2] McCarthy & Wilson (1932); Falconer, Epstein & Mills (1940); Wintrobe (1942); Schwartz & VonderHeide (1945); Aggeler *et al.* (1946); Schrumpf (1947).

[3] Gorrie (1940); Laird (1942); Lewis (1944); Nelson (1952).

[4] Russell & Page (1940); Goldbloom, Greenwald & Reinstein (1941); Losada & Fernandez (1942); Rosenfeld & Feldman (1942); Gorham, Propp, Schwind & Climenko (1943); Hurd & Jacox (1943); Kracke & Townsend (1943); Donaldson & Scarborough (1945); Geiger (1952); Bolton & Young (1953).

[5] Rosenthal (1928); Maritschek & Markowicz (1933); Posner (1937); Sturgis (1948); Grandjean (1948); Lincoln & Schmidt (1952); Bolton & Young (1953).

[6] Broch (1941); Nudelman, Leff & Howe (1948); Collins (1950); Moodie (1950); Hirsch & Dameshek (1950); Bigelow & Desforges (1952); Nussbaum & Shulman (1952); Bolton & Young (1953); Larson (1953); Barkham & Tocantins (1954).

[7] Hudson (1935); Lockie, Norcross & George (1947); Mettier, McBride & Li (1948); Heilskov (1950); Watson (1953).

The importance of establishing the diagnosis in this way is emphasized by two recent papers, one by Grant (1953) and the other by Grifoni *et al.* (1952). Both groups of workers reported anomalous results with in-vitro tests in cases of thrombocytopenic purpura suspected of being due to sedormid, but it is impossible to assess the significance of their findings as none of Grant's patients who were given sedormid after recovery developed purpura, and Grifoni's patient was not given a test dose.

In the case reports listed above, proof that the suspected drug had caused thrombocytopenic purpura was provided only in cases due to sedormid[8], arsenobenzol compounds[9], sulphonamides[10], quinine[11], quinidine[12], iodides (Davis & Saunders, 1946), chrysarobin (Peck *et al.* 1936), insulin (Strasser, 1935), digitoxin (Berger, 1952; Miescher & Ritter, 1953), ethylallylacetylurea (Graudal, 1949), Narcodorm (Amdrup, 1952), and antazoline (Ackroyd, 1954b). Thrombocytopenic purpura caused by foods would appear to be extremely rare, the only satisfactorily authenticated case being that described by Dutton (1938), in which the patient appears to have developed thrombocytopenic purpura after eating citrous fruits.

a. Thrombocytopenic Purpura due to Sedormid (Allylisopropylacetylurea)

i. Action of sedormid on the capillaries

The application of sedormid to the skin of some patients who have recovered from thrombocytopenic purpura due to this drug causes the appearance of petechial haemorrhages in the area of skin to which the drug is applied (Plate I, fig. A) (Ackroyd, 1949a). This reaction does not appear to be due to release of histamine, for the affected area shows neither hyperaemia nor wheal formation. The skin over the rest of the body is normal and the platelet count is unaltered. Sedormid has no action on the skin of controls.

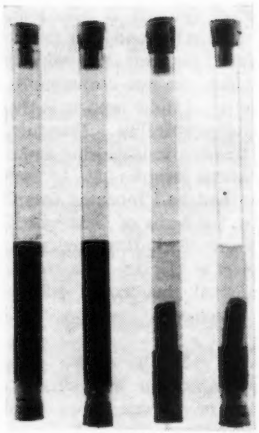
ii. Action of sedormid on the platelets

The addition of sedormid to the blood of sensitized patients causes a striking reduction in clot retraction (fig. 2) (Ackroyd, 1949a). This is caused by an abnormally rapid lysis of platelets by sedormid during coagulation, which can readily be demonstrated by observing the effect of sedormid on platelet-rich plasma allowed to clot in a haemocytometer chamber (Plate I, fig. B) (Ackroyd, 1949b).

Sedormid causes agglutination and lysis of platelets in the blood of sensitized patients, if this is kept fluid by the use of minimal quantities of anticoagulants (Plate II, figs. C and D) (Ackroyd, 1949c). Complement is fixed during platelet lysis (Ackroyd, 1951). The drug has no action on the blood of controls.

Sedormid causes agglutination and lysis of the platelets of normal individuals and of sedormid-sensitive patients when the platelets are suspended in the sera of sedormid-sensitive patients; but sedormid has no action on the platelets of sedormid-sensitive patients when the platelets are suspended in normal sera (fig. 3). These findings appear to show conclusively that the abnormality in the blood of patients who have recovered from sedormid purpura lies in the plasma or serum and not in the platelets (Ackroyd, 1951).

FIG. 2. ACTION OF SEDORMID ON THE CLOT RETRACTION OF THE BLOOD OF A SEDORMID-SENSITIVE PATIENT



Ackroyd (1949a) by permission of Clinical Science

A: blood + saturated solution of sedormid in saline. Clot retraction negligible

B: blood + saline. Clot retraction normal

Sedormid does not cause complement fixation if added to the sera of sedormid-sensitive patients alone. Platelets also are necessary. Red and white cells cannot replace platelets in this reaction. In the absence of complement, sedormid causes platelet agglutination, but does not cause platelet lysis (Ackroyd, 1951). These observations show that four factors are concerned in platelet lysis by sedormid: platelets, sedormid, complement, and the lytic factor in the sera of sensitized patients. It seems reasonable to assume that sedormid must combine with one of the other three factors. It is extremely unlikely that it combines with complement, because sedormid causes platelet agglutination in the absence of complement. Recent work (Ackroyd, 1954a) has shown that the union of sedormid with platelets and the lytic factor is remarkably loose. Both sedormid and the lytic factor can be washed away from platelets that have been suspended in the complement-free serum of a sensitized patient, to which sedormid has subsequently been added. If, however, the platelets in such an experiment are washed, not in saline, but in a saturated solution of sedormid in saline, the platelets remain attached to the lytic factor, thus showing that sedormid is necessary to fix the lytic factor to the platelets. If platelets are suspended in the complement-free serum of a sensitized patient, and sedormid is added, and the mixture is dialysed against saline, sedormid dialyses away from the mixture. When this happens the lytic factor becomes separated from the platelets, thus showing that sedormid probably acts as a link between platelets and the lytic factor, as shown in fig. 4. In no experiment has it proved possible to demonstrate any difference between the platelets of normal individuals and those of

[8] Loewy (1934); Falta (1936); Hadorn (1936); van Andel & Groen (1937); Lieberherr (1937); Markoff (1938); Falconer & Schumacher (1940); Moeschlin (1942); Aggeler *et al.* (1946); Ackroyd (1949a, 1949b, 1949c, 1951, 1952, 1954a); Miescher & Miescher (1952); Compston & Stewart (1953).

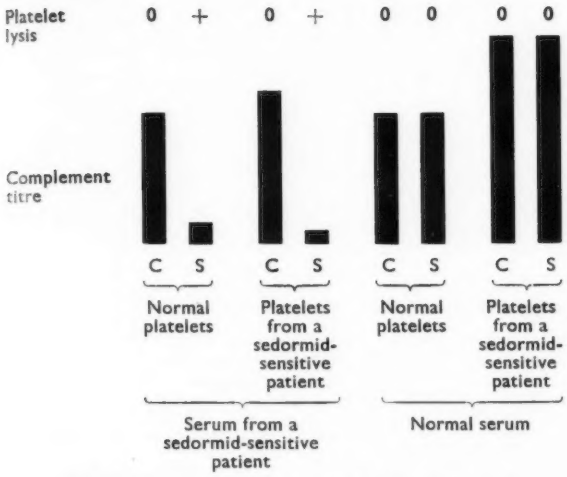
[9] McCarthy & Wilson (1932); Falconer *et al.* (1940); Schwartz & Vonder-Heide (1945); Aggeler *et al.* (1946).

[10] Hurd & Jacox (1943); Donaldson & Scarborough (1945); Bolton & Young (1953).

[11] Rosenthal (1928); Maritschek & Markowicz (1933); Posner (1937); Grandjean (1948); Sturgis (1948); Lincoln & Schmidt (1952); Bolton & Young (1953).

[12] Broch (1941); Nudelman *et al.* (1948); Collins (1950); Moodie (1950); Hirsch & Dameshek (1950); Bigelow & Desforges (1952); Nussbaum & Shulman (1952); Bolton & Young (1953); Larson (1953); Barkham & Tocantins (1954).

FIG. 3. PLATELET LYSIS AND COMPLEMENT FIXATION BY SEDORMID IN SUSPENSIONS OF PLATELETS FROM A NORMAL PATIENT AND A SEDORMID-SENSITIVE PATIENT IN THE HOMOLOGOUS AND THE HETEROLOGOUS SERUM


Platelet lysis
0 + 0 + 0 0 0 0
Complement titre
C S C S C S C S
Normal platelets
Platelets from a sedormid-sensitive patient
Normal platelets
Platelets from a sedormid-sensitive patient
Serum from a sedormid-sensitive patient
Normal serum

C: platelet suspension in serum was diluted with saline
S: platelet suspension in serum was diluted with a saturated solution of sedormid in saline

Ackroyd (1952) by permission of Progress in Allergy

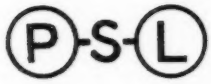
sedormid-sensitive patients. Sedormid separates equally readily from combination with either.

Confirmation of some of these findings in sedormid purpura has been reported by two groups of workers. Compston & Stewart (1953) demonstrated platelet agglutination and lysis by sedormid in the plasma, and complement fixation in the whole blood, of their patient, and were also able to show that platelet lysis depended on a factor in the plasma. Miescher & Miescher (1952) have described a case (Case I) in which the blood sedormid caused a reduction in clot retraction. They were unable to demonstrate any effect of sedormid on the platelets but they found that the addition of sedormid to the serum of this patient produced a precipitate. In this way, their case resembles the case of purpura due to antazoline described in section 3b.

iii. The immunological basis of sedormid purpura

The most probable explanation of these findings would appear to be that sedormid unites with platelets and, acting as a hapten, confers antigenic properties upon them. When the sedormid molecule, which supplies the determinant group, is removed, the platelets, no longer being antigenic, separate from the lytic factor (antibody). The chief difficulty in accepting this hypothesis is that sedormid can be removed so readily from combination with platelets and the antibody. This suggests that the compound formed by the union of sedormid and platelets must be extremely labile. For this reason, it is probably only weakly antigenic, for it is difficult to see how such a labile compound could remain in contact with the antibody-forming tissues long enough for it to be able to be more than a very weak stimulus to antibody formation (see Haurowitz, Tunca & Schwerin, 1943).

FIG. 4. PROBABLE MODE OF COMBINATION OF SEDORMID WITH PLATELETS AND THE LYTIC FACTOR IN THE SERA OF SEDORMID-SENSITIVE PATIENTS



P: platelets
S: sedormid
L: lytic factor

It is suggested that the very lability of this combination may explain why so few of those taking the drug develop purpura, for only those whose immune reactions are stimulated by this antigen will manufacture the antibody and so develop thrombocytopenia.

Thrombocytopenia in sedormid purpura is, therefore, probably the result of lysis of this platelet-sedormid antigen by antibody and complement, the antigen being formed whenever the drug is taken. The vascular lesion appears to be independent of thrombocytopenia, for capillary haemorrhages produced by patch-testing occur in the absence of any change in the platelet count. Now, the work of Bedson (1922) referred to above, showed that antiplatelet serum not only causes platelet lysis, but also damages the vascular endothelium. It seems reasonable, therefore, to suppose that the capillary damage in sedormid purpura is due to the action of the antibody that causes platelet lysis, and it may tentatively be suggested that sedormid may combine with the endothelial cells to form a further antigen, which then reacts with this antibody, so causing the vascular lesion which plays such an important part in the development of purpura.

b. Thrombocytopenic Purpura due to other Drugs

In thrombocytopenic purpura due to quinidine, the addition of the drug to the patient's blood or plasma, after recovery, has caused platelet agglutination (Bigelow & Desforges, 1952; Bolton & Young, 1953), and sometimes platelet lysis (Bigelow & Desforges, 1952; Barkham & Tocantins, 1954). Harrington (1954) has observed the agglutination by quinidine of a suspension of normal platelets in the serum of a sensitized patient. The drug has also caused a reduction in clot retraction (Larson, 1953; Barkham & Tocantins, 1954). These changes have been shown to be due to a factor in the patient's serum or plasma (Bigelow & Desforges, 1952; Larson, 1953; Bolton & Young, 1953; Harrington, 1954). In thrombocytopenic purpura due to quinine, the drug has caused platelet agglutination (Bolton & Young, 1953) and platelet lysis (Grandjean, 1948; Bolton & Young, 1953). Purpura due to sulphamezathine has been investigated by Bolton & Young (1953), who found that the drug caused platelet agglutination and lysis in the plasma of a sensitized patient. Stefanini (1954) has observed platelet agglutination by the causative drug in plasma from patients who had recovered from thrombocytopenic purpura due to Dormison (3-methylpent-1-yn-3-ol) and Chlor-Trimeton maleate; and platelet lysis has been produced by Narcodorm (Amdrup, 1952) and by Allymid (ethylallylacetylurea) (Graudal, 1949) in the plasma of patients who had recovered from thrombocytopenic purpura due to these drugs. The production of local purpura by the intradermal injection of *p*-aminosalicylic acid in a patient

who had developed thrombocytopenic purpura whilst taking this drug has been described by Le Melletier & Caulet (1952). Finally, a case of thrombocytopenic purpura due to antazoline (2-(*N*-phenyl-*N*-benzylaminomethyl)-imidazoline) (Ackroyd, 1954b) has been analysed in some detail. This condition appears to be very similar immunologically to sedormid purpura. The addition of antazoline to the blood caused platelet agglutination and lysis, and complement fixation, and also reduced clot retraction. The picture was, however, more complicated, for the addition of a dilute solution of antazoline in saline to the serum produced a precipitate which did not appear in the sera of controls. The precipitin responsible appeared to be identical with the antibody which caused platelet agglutination and lysis.

It seems clear from these reports that the fundamental mechanisms underlying sedormid purpura are similar to those underlying thrombocytopenic purpura due to other drugs although, as the condition can be produced by such widely different substances, it will be surprising if careful analysis of further cases does not reveal some immunological differences.

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MURAL THROMBOSIS IN ARTERIES

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1 The principle of the incorporation of surface deposits in arteries
2 Mural thrombi in relation to atherosclerosis
3 Fatty changes in mural thrombi
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The frequency of arterial thrombosis in man has never been fully appreciated because mural thrombi, which are the commonest form of the condition, are usually identified as atheroma, and their true nature consequently missed. This is remarkable, since Rokitansky[1], more than a hundred years ago, taught that atheroma was the product of mural thrombosis or, as he described it, an excessive deposition of fibrin on the lining membrane of the vessels. Had his teaching been followed, the present state of arterial pathology might have been more advanced than it now is, but the histologists of his day rejected it on the grounds that the lesions were in the vessel walls and not on them. They argued that since the changes were subendothelial in position they must represent some degenerative or inflammatory condition of the intima, and in doing so they failed to recognize one of the most important principles of arterial pathology.

1. The Principle of the Incorporation of Surface Deposits in Arteries

When fibrin is deposited on the internal surface of an artery it becomes covered with endothelium so that it is incorporated in the vessel wall, and any further changes that occur in it appear as if they were in the vessel wall rather than on the surface. The principle was first deduced from a study of human material (Duguid, 1946, 1948), but experimental proof has been provided first by Harrison (1948) and later by Heard (1952) and McLetchie (1952). These authors showed that autogenous fibrin particles injected into the veins of rabbits become lodged as emboli in the pulmonary arteries and are in due course converted into fibrous thickenings of the vessels. Similar experiments have been carried out in my laboratory by Dr Lichtenberger, and I have his permission to show some of the results.

Fibrin clots from the rabbit's own blood were broken into small particles and reinjected into the ear veins. In most instances repeated injections were given at intervals of several days, so that most of the fragments subsequently found were of uncertain age, but nevertheless the successive stages of the process could be followed. Plate III (fig. E) shows a clump of particles in a pulmonary artery five or six days after injection, covered by endothelium and fixed by it to the vessel wall. The fibrin is now homogeneous and hyaline in appearance and no longer gives the specific staining reactions of fibrin. Plate III (fig. F) shows a similar mass of two months' standing, incorporated in the vessel wall so as to form a hyaline thickening of the intima. It will be noted that in addition to the covering endothelium there is now a well-marked subendothelial layer of fibrous tissue so that the mass appears as if it were quite deeply embedded in the intima. After several months, deposits of this kind are usually converted into fibrous thickenings of the vessels.

These illustrations make it clear that the endothelium of an artery is an adjustable membrane, and that changes which are subendothelial in position need not necessarily have originated in that position. They also show that when fibrin is incorporated in the wall of an artery it becomes so changed in appearance as to be easily mistaken for some kind of degeneration of the intimal tissues.

2. Mural Thrombi in Relation to Atherosclerosis

With the above principle in mind one can recognize a similar sequence in the development of certain arterial lesions in man, especially atherosclerosis. In human arteries mural thrombi vary from microscopic layers of fibrin or platelets up to masses of clot large enough to occlude the lumina, and their fate is much the same as that of the experimental emboli. When newly formed they are usually ragged and project irregularly on to the lumina of the arteries (Plate III, fig. G), but later, under the influence of the blood pressure, they become smoothed out (Plate III, fig. H) and more or less moulded to the shape of the vessel walls. Within a short time, probably 48 hours if we can judge by the experiments carried out on small animals by Dr G. Williams in my department, a layer of endothelium spreads over them from the surrounding intima, and this is followed by the formation of a subendothelial layer of connective tissue, so that the thrombi now appear as intimal thickenings. Almost from the outset their appearance changes, the fibrin which is at first fibrillar, as in Plate III (fig. G) becomes granular or flocculent, and later homogeneous, while at the same time it loses its specific staining properties, and takes up a variable pink with eosin. In red thrombi the corpuscles fuse with the fibrin into more or less homogeneous masses, but some of the leucocytes tend to retain their nuclear staining and for long remain prominent. One is sometimes tempted to suspect that these cells may survive indefinitely and subsequently take part in the process of organization. Finally, fatty changes make their appearance in the thrombi and the condition is then identified as atherosclerosis.

3. Fatty Changes in Mural Thrombi

Fatty changes first make their appearance as fine sprinklings of lipid droplets amongst the thrombotic elements, and they soon become the most prominent feature. They are usually most plentiful in red thrombi which contain much whole blood with relatively little fibrin, and it is in such formations

[1] The most easily accessible record of Rokitansky's teaching on this subject is his *Manual of pathological anatomy*, translated by George E. Day, London: Sydenham Society, 1852, vol. 4, pages 261–272.

MURAL THROMBOSIS IN ARTERIES

(FIGS. E—M)

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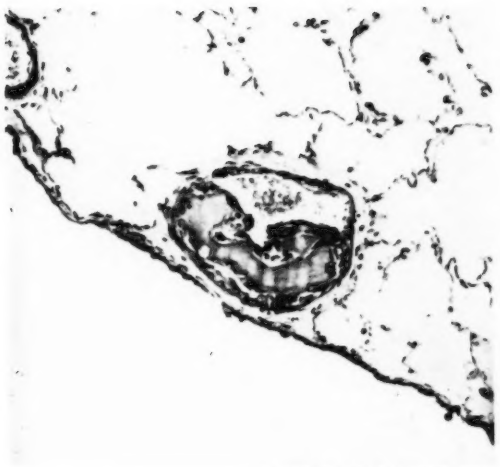

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FIG. E. Experimental pulmonary embolism in rabbit: fibrin embolus of five to six days' standing, lodged in a pulmonary artery. It consists of several fragments covered by endothelial cells and attached by them to the vessel wall. The fragments are hyaline in appearance and no longer show the specific staining reactions of fibrin

(Paraffin section: haemalum and eosin; magnification × 125)

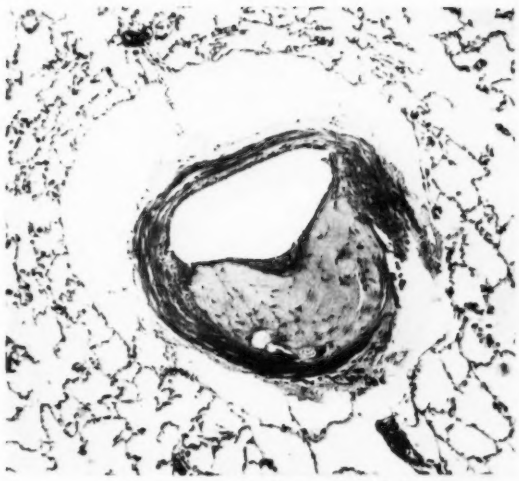

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FIG. F. Experimental pulmonary embolism in rabbit: fibrin embolus of two months' standing, incorporated in the wall of a pulmonary artery. In addition to the covering endothelium there is a subendothelial layer of fibrous tissue, so that the mass appears to be embedded in the intima. Early stages of organization are represented by connective tissue cells scattered through the mass and a capillary penetrating its base

(Paraffin section: haemalum and eosin; magnification × 125)

FIG. G. Mural thrombi in human aorta: two distinct layers of deposit are seen in the intimal surface—the more superficial, and recent one, composed of loose fibrin, and the deeper consisting of laminae of the hyaline substance which is typical of older deposits. Early organization can be seen at the base, consisting of a penetration by connective tissue cells between the laminae of hyaline substance and the formation around them of layers of connective tissue

(Frozen section: haemalum; magnification × 50)

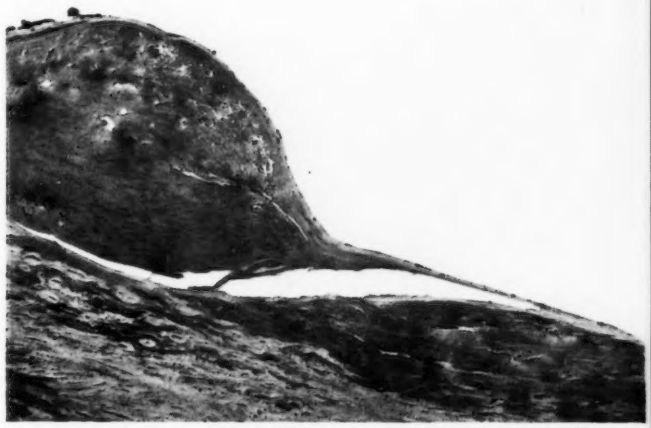

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FIG. H. Mural thrombi in human aorta: two hyaline masses representing old fibrin deposits are seen in the surface of the intima, the one partly overlapping the other. The more superficial one is covered with endothelium and also by a thin subendothelial layer of fibrous tissue which is slightly less darkly stained. A similar layer of fibrous tissue separates the two deposits where they overlap and presumably represents an earlier subendothelial formation occurring before the time of the second deposit. At the surface on the extreme left there are some fragments of fibrin and cells representing a still more recent deposit

(Frozen section: haemalum; magnification × 70)

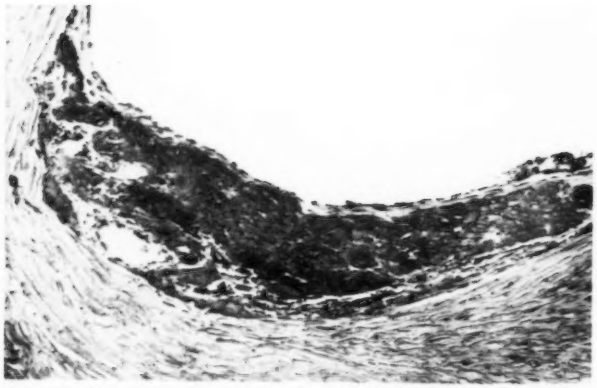

FIG. J. Organizing mural thrombus in human aorta: the darker staining material is a fibrous thrombus which has been incorporated in the intima. On the left it is penetrated by capillaries around which there are zones of new-formed fibrous tissue. Other sections from the same lesion showed similar capillaries connecting with the lumen

(Frozen section: haemalum; magnification × 45)

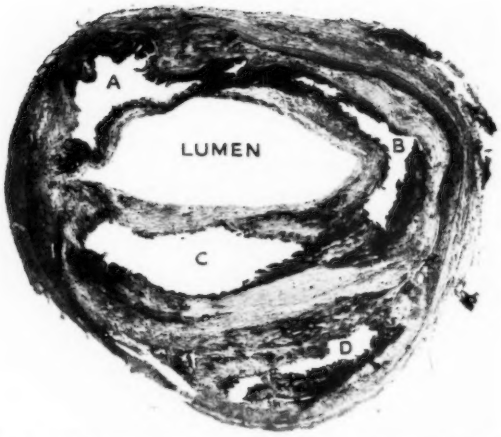

FIG. K. Atherosclerotic coronary artery: the apparent tears marked A, B, C and D are atheromatous foci from which the softened material has been washed out in the process of mounting. C and D represent successive mural thrombi, one superimposed on the other, and both undergoing partial organization as well as softening

(Frozen section: haemalum and Sudan IV; magnification × 15)

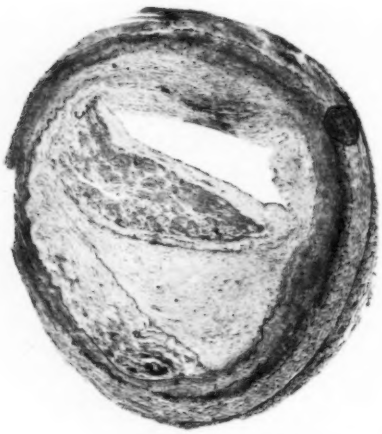

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FIG. L. Atherosclerotic coronary artery: three successive deposits are clearly demarcated, the outer one completely organized except for the dark stained focus of partly calcified atheroma at the lower part of the section, the intermediate one entirely converted into fibrous tissue, and the inner one still containing unorganized fibrin which is also stained darker than the surrounding fibrous tissue

(Frozen section: haemalum and Sudan III; magnification × 18)

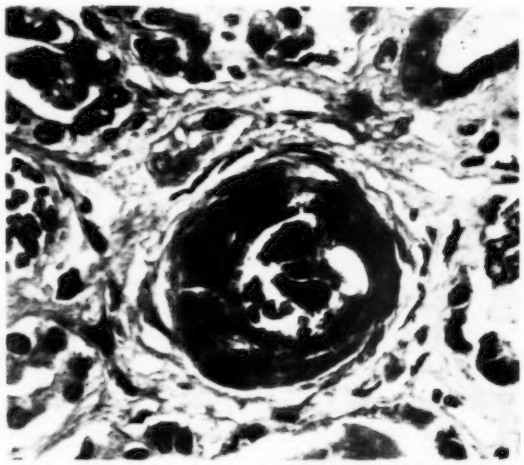

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FIG. M. Hyaline arteriolosclerosis in hypertensive nephrosclerosis: the hyaline substance, which shows darker than the rest of the tissue, is partly free in the lumen and partly coating the walls. There is no endothelial lining and the original vessel wall is represented by the ring of nucleated cells encircling the hyaline mass

(Paraffin section: haematoxylin and eosin; magnification × 470)

that softening occurs, with the formation of atheromatous cavities in the vessel walls (Plate IV, fig. J).

It is undoubtedly the occurrence of fats in mural thrombi that has led to the difficulty in their identification. We have been taught to regard fatty changes as signifying a degeneration of the vascular tissues, and the idea of their being products of surface deposits has not as a rule entered our minds. Moreover we have not in the past associated fatty change with thrombosis, because our conception of vascular thrombosis has been based mainly on the study of arteries or veins that have been totally occluded by thrombi, in which case fatty changes are not usually seen. This is perhaps due to a more rapid organization. Mural thrombi, especially in the larger arteries, are exposed to the full force of the blood pressure and to the movements of pulsation, and organization appears to be retarded, or in many instances brought to a standstill, so that time is allowed for degeneration to occur. The same conditions apply to cardiac thrombi, including the vegetations of chronic endocarditis, where—although the fact is seldom recorded—fatty changes are equally common.

4. Organization of Mural Thrombi

Sometimes the large mural thrombi show the classical form of organization (Plate IV, fig. J), with capillaries penetrating either from the lumen or from the subjacent vessel walls—and this no doubt accounts for the vascularization of the intima so often cited as a characteristic feature of atherosclerosis—but the majority are relatively small and in them organization is a simple process in which cells alone are the active agents. In the subendothelial zone a layer of fibrous tissue is formed (Plate III, figs. F, H and Plate IV, fig. J), evidently from the endothelium, while in the deeper zones spindle cells from the subjacent intima invade the hyaline substance and form centres of connective tissue development. As the connective tissue increases it gradually replaces the hyaline substance, but before this process is completed it is usually obscured by fatty change.

Some of the most striking examples of retarded organization are seen in the atheromatous softenings to which I have already referred (Plate IV, fig. K). In them cavities are formed which are apparently beyond the powers of the organizing process to penetrate, and although zones of organization may form around these cavities, sometimes with vascularization, their centres remain as more or less permanent foci of degeneration.

5. Recurring Mural Thrombosis

As I have explained, the formation of a covering layer of endothelium and a subjacent layer of connective tissue has the effect of burying mural thrombi in the intima, but this effect is carried further when fresh surface deposits are superadded. As may be seen from some of the illustrations, mural thrombosis is a recurring condition, and successive layers of fibrin tend to be deposited one on top of another and in turn organized, so that cumulative thickenings of the intima are produced (Plate IV, fig. L). This, in medium or small vessels such as the coronaries, results in progressive narrowing of the lumina, and constitutes the danger in atherosclerosis, but it must be realized that the narrowing of arteries by thrombosis can be a temporary affair. Mural thrombi under the influence of the blood pressure become compressed and reduced in size, so that a mass which may at first almost occlude the lumen becomes flattened out and, if completely organized, forms a relatively thin layer of fibrous tissue. In fact we frequently find that arteries which have obviously been thrombosed are actually dilated, the fibrous thickenings of their walls having interfered with their elasticity and rendered them liable to permanent stretching. The narrowing of arteries by mural thrombosis has therefore to be looked on as both a regressive and progressive process, regressive in that each individual thrombus, given time, shrinks; but progressive in that thrombosis tends to recur, so that it is in rapidly recurring thrombosis that the danger lies.

It is not suggested that all atherosclerosis is thrombotic in origin—for there are fatty changes to be found in the young aorta which are undoubtedly the outcome of other processes (Duguid, 1954)—but it can be shown that most of the features we regard as characteristic of the advanced atherosclerosis, including fibrous thickening, hyaline and fatty degeneration, atheromatous softening and vascularization of the intima, can be the products of mural thrombi. In fact it is difficult to explain them in any other way; and the further we inquire into the histology of atherosclerosis the more frequent an occurrence does arterial thrombosis seem to be.

6. Thrombosis in Other Forms of Arterial Disease

The principle of incorporation of surface deposits provides a new approach to arterial pathology, and there are many lesions other than atherosclerosis which have to be reviewed in the light of it. One which suggests itself is the so-called hyaline arteriosclerosis seen in the kidneys, spleen and other organs in cases of hypertension and diabetes mellitus. The accepted pathology of this condition seems paradoxical because, whereas the lesions are regarded as degenerative or necrotic, they are associated with narrowing of the vessel. Arterioles have to withstand a high internal pressure of blood, and degeneration or necrosis would be expected to cause them to give way and dilate rather than become narrowed. With this in mind the histology has recently been reviewed (Duguid & Anderson, 1952), and evidence found which did in fact seem to suggest that the thickenings originated as deposits of fibrin or other solid matter in the lumina of the arterioles.

In the typical hypertensive arteriolosclerosis a layer of hyaline substance, usually with some fatty change, surrounds the lumen of the arteriole, displacing the medial coat outwards. In the majority of instances its inner surface is lined with endothelium so that it has all the appearance of an intimal thickening, but if one searches carefully through almost any severely affected kidney or spleen one will usually find exceptions in which the hyaline substance is exposed to the blood stream (Plate IV, fig. M). In such instances the internal surface is usually ragged, and there may be loose fragments of hyaline or sometimes fibrinous substance more or less mixed with the blood, while sometimes the lumina are practically plugged with the same substance. Such formations are best seen in malignant nephrosclerosis, where the substance concerned is usually quite clearly identifiable as fibrin. What the hyaline substance may be, whether fibrin, platelets, or simply an albuminous coagulum derived from the blood plasma, is not so easy to decide, but there is a tendency at the present time to identify formations of this kind as platelets:

for example, in the platelets thrombosis syndrome—apparently for no other reason than that they are hyaline. When it is recalled, however, that the fibrin injected into the veins of rabbits (Plate III, figs. E, F) forms hyaline emboli, it will be realized that histological appearances are not a very safe basis for such conclusions.

These findings are by no means decisive but they show that hyaline arteriolosclerosis could be deposition of fibrin or other coagulum in the lumina of the arterioles just as well as a degeneration in their walls, and such an explanation would be more in keeping with the narrowing than is the degeneration theory.

7. Frequency of Mural Thrombi

There are no doubt other arterial lesions to which the principle of the incorporation of surface deposits may apply, such as, for example, the peculiar vascular formations seen in glioblastomata and other cerebral tumours (Storring & Duguid, 1954) and some of the lesions in periarteritis nodosa (Duguid, 1955), but a discussion on them is beyond the scope of this communication. The evidence in atherosclerosis alone is enough to show that arterial thrombosis must be a far commoner occurrence than we have been led to imagine. We have been taught to regard it as an occasional complication of atherosclerosis, but if we are right in our interpretations we must now look on it as an important factor in the pathogenesis of that condition, and, since atherosclerosis is almost universal in adult life, mural thrombosis must be a commonplace event.

ACKNOWLEDGEMENT

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MODE OF ACTION OF COUMARIN DRUGS

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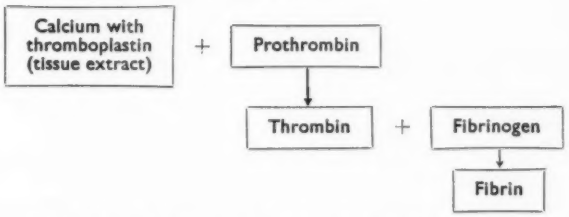
1 Discovery and development
2 Mechanism and site of action
3 The coumarin drugs and the coagulation mechanism
  a The importance of blood thromboplastin
  b Analysis of the defect caused by the coumarin drugs
  c The disturbance of coagulation resulting from therapy
4 Conclusions
References

Whatever may be the final assessment of the role of the coumarin drugs in the therapeutic management or prevention of thrombo-embolic disease, it is of some importance that there be an understanding of the mechanism by which these drugs interfere with coagulation. There is some evidence that the action is not restricted to changes in blood coagulation. Kubik & Wright (1951) have concluded that adequate therapy with coumarin compounds is beneficial in promoting recanalization of arteries and veins in animals where experimental occlusion and thrombus formation have occurred. There are some reports of interference with capillary physiology. These effects may be secondary phenomena resulting from the interference with coagulation; on the other hand they may represent independent actions of the drugs.

It is not the purpose of this account to deal with these other possible effects. At present coumarin drugs are used therapeutically only as anticoagulants, and the remarks are limited to their action in producing changes in coagulation demonstrable in vitro. The evidence of interference with clotting processes in vivo has been reviewed elsewhere (Brown & Douglas, 1952).

1. Discovery and Development

About thirty years ago a haemorrhagic disease of cattle was recognized as being due to feeding on spoiled sweet clover hay, and this condition became known as "sweet clover disease". The identification of dicoumarol, the causal agent of the disease, by Link and his associates in 1940, has been one of the outstanding achievements of biological and biochemical research in recent years (Link, 1944). Thereafter there was clinical trial of dicoumarol as a therapeutic agent in human thrombo-embolic disease. Since the preliminary report by Butt, Allen & Bollman (1941), there has been a widespread interest in the pharmacology of the coumarin drugs. There has followed the development of coumarin derivatives and of allied compounds. When the drug dicoumarol was first used, the readily identified change in the patients' coagulation mechanism was a prolongation of the clotting time as determined by the one-stage test of Quick. This test, which had been introduced in 1935, was based on the "classical" theory of blood coagulation (Quick, 1935). This theory postulated that prothrombin was converted to thrombin under the influence of "thromboplastin" (or "thrombokinase") and free calcium ions, the thrombin then interacting with fibrinogen to produce fibrin. It became generally accepted that extracts of tissues such as brain owed their coagulant activity to their content of thromboplastin, so that the terms "thromboplastin" and "tissue extract" are still often taken to be synonymous.



The principle of Quick's test was that, on the addition to plasma of an excess of tissue extract and calcium, the resulting clotting time would be controlled by the concentration of only one factor—prothrombin. The test was referred to as the one-stage clotting time, or the one-stage prothrombin time. It was because of this easily demonstrable effect that these drugs were said to produce "hypoprothrombinaemia".

The drugs of the coumarin group that are readily available in Great Britain are shown in Table I. The effect of each on the one-stage prothrombin time, using brain as tissue extract, is also shown in Table I.

It will be observed that after the administration of a single dose there is variation in the time of onset of effect, and the duration of this effect once induced. At one end of the scale is Tromexan with the most rapid onset, and short-lived effect; at the other extreme is Marcoumar with a slow induction time, and a very prolonged response once induced.

2. Mechanism and Site of Action

When given by mouth, dicoumarol is slowly and erratically absorbed from the gastrointestinal tract, and thereafter the drug is gradually metabolized to unknown transformation products (Weiner, Shapiro, Axelrod, Cooper & Brodie, 1950). For each subject there is a threshold plasma value, which must be reached before a detectable "hypoprothrombinaemic" response is elicited. This is generally between 0.5 mg. and 1.0 mg./100 ml. There is considerable lag between maximal plasma level and maximal prothrombin response, and no close relationship exists between the plasma dicoumarol level and the effect on prothrombin activity in different subjects.

The site of action of these drugs has always been assumed to be the liver and there is some experimental and presumptive evidence that this is so. The experiments of Lupton (1947) have shown that, after dicoumarol is administered to rats, the prothrombin activity of blood perfused through the liver is not increased, whereas similar perfusion of the liver of untreated rats is associated with a rise in prothrombin activity of the perfusion fluid. In experimental animals, Lee, Trevoy, Spinks & Jaques (1950)—using dicoumarol labelled with radioactive carbon—demonstrated that about 10% of the

TABLE I. DRUGS OF THE COUMARIN GROUP READILY AVAILABLE IN GREAT BRITAIN, AND THEIR THERAPEUTIC EFFECTS

Chemical name	Name of drug	Supplied by	Latent period (hr.)	Approximate time required to produce therapeutic "hypoprothrombinaemia" (hr.)	Approximate time required for prothrombin time to return to normal after single dose (days)
Methylene-bis-3-(4-hydroxycoumarin) (dicoumarol)	Dicoumarol Dicoumarin	Herts Pharmaceuticals Ltd. Organon Laboratories Ltd. Ward, Blenkinsop & Co. Ltd. Light & Co. Ltd.	24–48	48–72	4
Bis-3-(4-hydroxycoumarinyl)-ethyl acetate (BOEA)	Tromexan	Pharmaceutical Laboratories Geigy Ltd.	8–12	18–36	1–2
3 : 4-(2′-Methyl-2′-methoxy-4′-phenyl) dihydropyranocoumarin (cyclocoumarol)	Cumopyran	Abbott Laboratories Ltd.	18–24	24–48	8
3-(1′-Phenylpropyl)-4-hydroxycoumarin (phenyl propyl hydroxycoumarin)	Marcoumar	Roche Products Ltd.	24–48	48–72	7–14
Phenylindanedione	Dindevan Indema	Evans Medical Supplies Ltd. Imperial Chemical (Pharmaceuticals) Ltd.	18–24	24–36	2–3

radioactivity became fixed in the liver, and that this was owing to unchanged dicoumarol.

The structural resemblance of dicoumarol to vitamin K led to the suggestion (Woolley, 1947) that dicoumarol acts by competitive interference with vitamin K and that, because of some structural similarity, it may replace this substance in an enzyme system involved in the formation of those coagulation factors rendered deficient by the administration of the drug. Direct proof of this has not been presented, but there is some suggestive evidence: for example, detailed assessment of the coagulation defect in vitamin K deficiency shows that it is the same as that produced by the coumarin drugs (Douglas, unpublished observations, 1954); administration of vitamin K_1 is able to prevent or reverse the action of these drugs (Douglas & Brown, 1952).

3. The Coumarin Drugs and the Coagulation Mechanism

a. The Importance of Blood Thromboplastin

According to the classical theory of blood coagulation, prothrombin conversion is controlled by thromboplastin and calcium, and the term thromboplastin has been accepted by many as synonymous with tissue extract. But, as will be described presently, the term is used here in a more specific sense, referring to the agent directly responsible for prothrombin conversion. When blood is collected by clean venepuncture and delivered to two glass tubes, one of which contains some tissue extract, the blood mixed with tissue extract coagulates in a few seconds, whereas the blood in the other tube requires several minutes to clot. The process involved in prothrombin conversion under the influence of the tissue extract is referred to as the extrinsic thromboplastin-forming mechanism. The blood which has been collected without any possibility of tissue contamination, and allowed to clot in the plain tube, has coagulated under the influence of its own thromboplastin—the intrinsic thromboplastin-forming mechanism. This intrinsic mechanism has been shown to produce a very powerful thromboplastin (Biggs, 1952; Biggs, Douglas & Macfarlane, 1953a). The long clotting time of whole blood is due to a delay in the formation of this potent blood thromboplastin. Tissue damage is an inevitable accompaniment of injury, but the relative importance of the extrinsic and intrinsic mechanisms in physiological haemostasis remains uncertain. For example, in haemophilia there is a readily demonstrable defect in intrinsic thromboplastin formation, but brain extract, prepared from a haemophiliac who died of haemorrhage, was just as powerful in promoting the clotting of haemophilic plasma as that prepared from normal brain (Brown, unpublished observations, 1951). It is necessary, therefore, to consider to what extent the coumarin drugs interfere with intrinsic as well as extrinsic thromboplastin formation, in view of the definite possibility that the former is the more important in physiological haemostasis.

One of the earliest indications of possible interference with intrinsic thromboplastin production came from an observation involving comparison of the whole-blood clotting time in plain and in silicone-treated tubes. The blood of patients under coumarin therapy had a normal whole-blood clotting time, when delivered into glass tubes. When the clotting time was estimated in silicone-treated tubes, it was markedly prolonged as compared with the normal in such tubes. These were tests of clotting efficiency performed under conditions where only the intrinsic thromboplastin mechanism was called into action.

Despite the recent realization of the importance of the blood's own thromboplastin, it is my intention to give some

account of the effect of coumarin therapy on the extrinsic mechanism, before going on to the intrinsic.

This sequence has been necessary to enable the introduction, in chronological order, of certain components, which were initially discovered in relation to tissue extracts but subsequently were realized to be required for blood thromboplastin formation also. Thromboplastin, whether it be derived wholly from the blood, or by the action of tissue extracts, causes prothrombin conversion, and any interference with either mechanism of production results in interference with blood coagulation.

Apart from the assay of prothrombin itself, the tests to be described measure the efficiency of the extrinsic or intrinsic systems in causing conversion of prothrombin to thrombin.

b. Analysis of the Defect Caused by the Coumarin Drugs

i. The action of tissue extracts

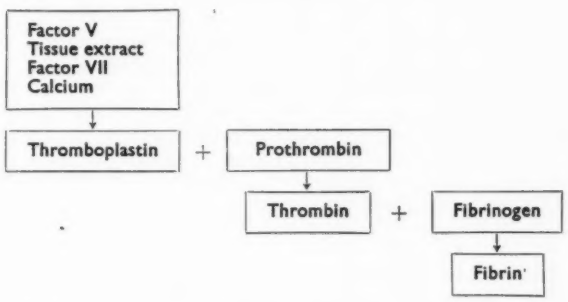
As already mentioned, the result of Quick's one-stage " prothrombin " test was originally considered to be dependent on quantitative variation in prothrombin. More recently, however, and largely as a result of the observations of Owren (1947), it has become evident that at least one other factor, the so-called Factor V, was concerned in the conversion of prothrombin under the influence of tissue extract. It was in the light of this discovery that the nature of the action of the coumarin drugs was reinvestigated, and it was soon established that the major deficiency in coumarin plasma was of another coagulation component, which came to be known as Factor VII. The extent of the deficiency of this factor required to be established. In addition, since the one-stage test did not reflect change in prothrombin concentration, methods had to be designed for the measurement of prothrombin itself—the precursor of thrombin.

Owren's discovery of Factor V was dependent on the study of a patient with a haemorrhagic disorder who had a prolonged one-stage prothrombin time. It was found that plasma which had been treated with an inorganic precipitate to remove prothrombin was able to correct the one-stage clotting time in Owren's case. The technique of adsorption on inorganic precipitates is a time-honoured and simple method of plasma fractionation used in the study of blood coagulation. The adsorbent that is in common use in Great Britain is aluminium hydroxide. The plasma remaining after adsorption by aluminium hydroxide contains anti-haemophilic globulin and Factor V. Similar principles were applied to the defect in coumarin plasma. It was soon established that coumarin plasma did not lack the easily identified Factor V and, according to the theory as it stood, this plasma was believed to be deficient in prothrombin. Further investigation rapidly upset this belief. When clotted whole blood was kept at 37° C. for one hour, its resulting serum contained no prothrombin, but this serum was able to produce marked correction of the one-stage test of coumarin plasma. Plasma was collected from a patient who was on the third day of Tromexan therapy. The one-stage clotting time of this patient's plasma was 56 seconds and that of a control simultaneously and identically handled 15 seconds. When one-tenth part of normal serum was added to the patient's plasma, the one-stage clotting time of the mixture was 16 seconds. In this manoeuvre, serum containing no prothrombin was able to correct the defect in the patient's one-stage clotting time. But the patient's plasma was known not to lack Factor V. From this simple observation came the realization that there was yet another component involved in the reactions resulting in blood coagulation occurring under the influence of tissue extract. The term Factor VII has been adopted for this coagulation factor because Koller, Loeliger & Duckert (1951), who introduced this term, made the most illuminating study of the phenomenon. The features of Factor VII are summarized in Table II.

TABLE II. PROPERTIES AND FUNCTION OF FACTOR VII AND PROTHROMBIN

Factor VII	Prothrombin
i. Present in the fraction of plasma or serum removed by inorganic precipitates	i. Present in the fraction of plasma removed by inorganic precipitates
ii. Not consumed during clotting, and therefore present in serum	ii. Consumed during clotting and in consequence not present in serum
iii. Diminished in concentration to considerable extent by administration of coumarin drugs	iii. Diminished in concentration to a limited extent by the administration of coumarin drugs
iv. Deficiency readily detectable by the one-stage " prothrombin " test	iv. Deficiency not readily detectable by the one-stage " prothrombin " test
v. Forms an integral part of thromboplastin formation	v. The precursor of thrombin

At this stage in the development of our knowledge of blood coagulation, it was realized that prothrombin could not be satisfactorily converted to thrombin by tissue extract alone. In addition to tissue extract, the two plasma components called Factor V and Factor VII were required. Biggs, Douglas & Macfarlane (1953c) have produced evidence to show that the tissue extract, Factor V and Factor VII interact to produce an agent which converts prothrombin directly. Since, in the classical theory of coagulation, the activator of prothrombin was called " thromboplastin ", this term was retained to describe the product of this interaction. Thus tissue extracts do not contain thromboplastin in this sense, but are capable of producing it in conjunction with calcium and the Factors V and VII derived from the blood. This system, in which tissue extract is an essential component, is referred to as the extrinsic thromboplastin-forming mechanism, thus:



The fraction of serum containing Factor VII can be separated by adsorption on aluminium hydroxide and subsequent elution. When these fractions are prepared from normal serum and coumarin serum, and incubated with tissue extract

and Factor V to produce thromboplastin, the defective reaction of the coumarin fraction is readily demonstrated (Douglas, unpublished observations, 1952).

ii. Intrinsic or blood thromboplastin formation

Subsequent to the discovery of Factors V and VII and their relationship to tissue extract, the blood's own thromboplastin was extensively investigated (Biggs *et al.* 1953a, 1953b, 1953c; Biggs & Douglas, 1953b). Factors V and VII were found not only to be required for thromboplastin formation in the presence of tissue extracts, but to be essential components for blood thromboplastin formation, in addition to antihaemophilic globulin, platelets, and the Christmas factor. Antihaemophilic globulin is the missing component in haemophilia, and the Christmas factor the deficiency in Christmas disease. The two systems can be compared as follows:

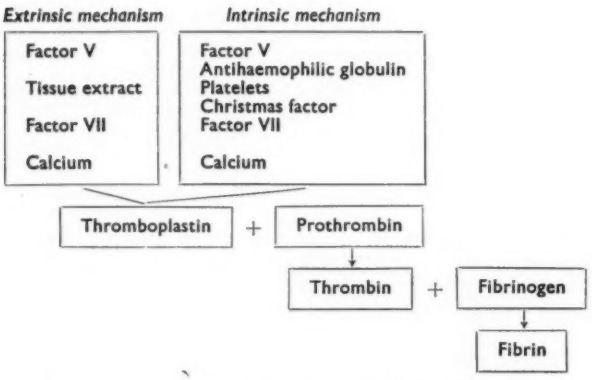
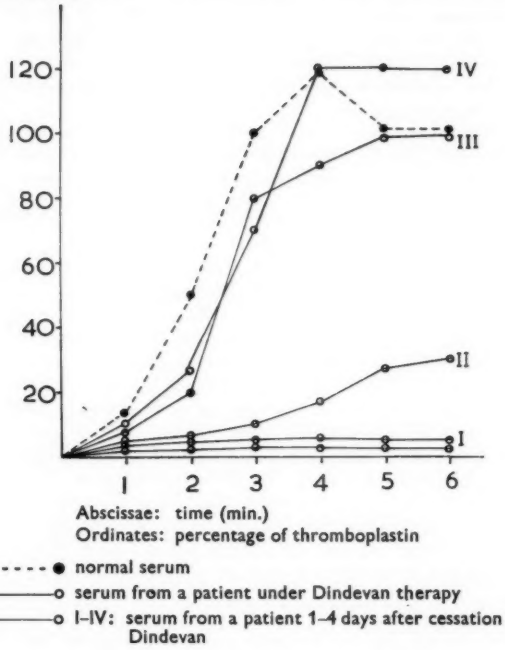


FIG. 1. THROMBOPLASTIN GENERATION: SERUM FROM A PATIENT UNDER DINDEVAN THERAPY IS COMPARED WITH NORMAL SERUM; CURVES I–IV SHOW THE INCREASING POWER OF THE SERUM TO FORM THROMBOPLASTIN ON THE SUCCESSIVE DAYS AFTER CESSATION OF THERAPY



In haemophilia, thrombocytopenia and Christmas disease, the plasmas have normal one-stage clotting times. Tissue extract in the extrinsic system is equivalent to antihaemophilic globulin, platelets and the Christmas factor in the intrinsic. These principles have been employed in the design of the thromboplastin-generation test described elsewhere in this issue.[1] When $Al(OH)_3$-treated plasma (containing Factor V and antihaemophilic globulin), platelets, and serum (containing Christmas factor and Factor VII) are incubated together in the presence of calcium, a powerful thromboplastin develops. By making various permutations of these components derived both from the normal subject and from the patient under investigation, the site of a defect in the patient's intrinsic thromboplastin formation can be traced.[2] When serum from patients under therapy with the coumarin drugs is substituted for normal serum, there is defective thromboplastin formation. This is illustrated in fig. 1, which also shows the gradual return to normal values over the four days after stopping Dindevan therapy.

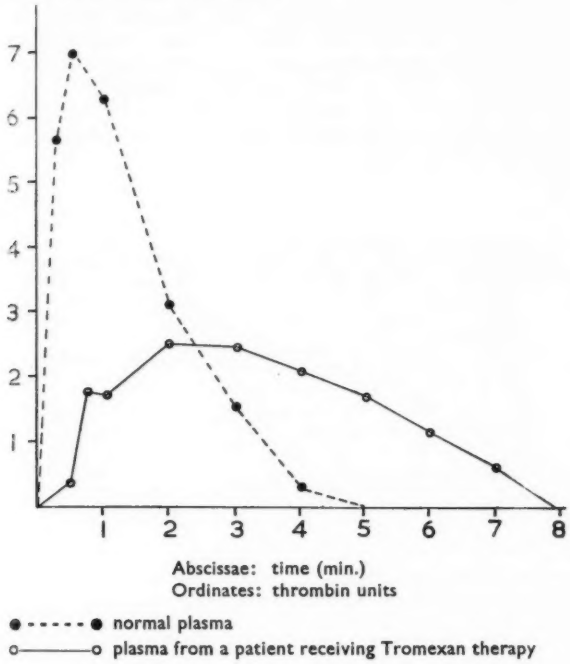
iii. Prothrombin

When it was realized that the deficiency in Factor VII was able to cause very significant prolongation of the one-stage test, it remained to determine whether there was any deficiency in prothrombin itself in plasma from patients receiving drugs of the coumarin group. The properties and function of prothrombin in comparison with those of Factor VII are shown in Table II. Until recent years there was no method in existence which could be considered to give a true quantitative assay of prothrombin. Two methods have now been provided for this purpose (Biggs & Douglas, 1953a; Douglas & Biggs, 1953). These are two-stage techniques dependent on the generation of thrombin, which is measured by the clotting time of fibrinogen subsequently exposed to its action.

An example of the first method (Biggs & Douglas, 1953a) is shown in fig. 2. A mixture is made of plasma, brain extract, and calcium, and the pattern of thrombin generation is followed by the serial transference of equal volumes of the mixture into fibrinogen. It will be noted that the plasma of a patient receiving Tromexan produces thrombin more slowly than does the normal, but that this production continues for a much longer period of time than in the normal. The slow initial production of thrombin explains the long one-stage "prothrombin" time. The appearance of clot which forms the end-point of Quick's test is controlled by the speed of thrombin production rather than by the amount. The prothrombin can be measured by comparing the area enclosed by these curves—the patient against the normal (fig. 2). The second method (Douglas & Biggs, 1953) employs a technique of separation of the prothrombin from antithrombin, and its subsequent activation by brain extract and calcium. By the use of these methods, the mean prothrombin content of a large series of random specimens from patients being treated with Tromexan was 65 % (Douglas, unpublished observations,

[1] See Biggs, p. 5 of this number of the Bulletin.—Ed.
[2] See Pitney & Dacie, p. 11 of this number of the Bulletin.—Ed.

FIG. 2. TWO-STAGE PROTHROMBIN ASSAY: THE PATTERN OF THROMBIN GENERATION BY NORMAL PLASMA IS COMPARED WITH THAT OF PLASMA FROM A PATIENT RECEIVING TROMEXAN THERAPY



1952). The prothrombin content of the plasma of patients receiving other members of the group of drugs has not been assayed in sufficient subjects to reach any final conclusion; there is some evidence to suggest that the longer the duration of action of the coumarin drug, the lower will be the prothrombin concentration of the plasma. It is possible that Tromexan, having the shortest action time of this group, has the least effect on the prothrombin content.

c. The Disturbance of Coagulation Resulting from Therapy

The one-stage test remains the sheet anchor of control of therapy with this group of drugs. Even though the one-stage test does not assay what it was originally thought to measure, nevertheless it is very sensitive to the decreased concentration of Factor VII which is the main defect in coumarin therapy. The thromboplastin-generation test, though a very sensitive technique, is too elaborate for routine control of anticoagulant therapy. When normal plasma is diluted with saline, and the one-stage test performed on these dilutions, a curve of percentage in reference to the undiluted sample can be prepared. In the case of Tromexan the prothrombin content is relatively so high that any deficiency is unlikely to be contributing significantly to the prolongation of the one-stage clotting time. Biggs & Douglas (1953a) have described a patient in whom the one-stage test was little altered although the prothrombin concentration was only 10% of normal. The Factor VII content in this patient's plasma was normal. In these circumstances the clotting time in the one-stage test during Tromexan therapy, when expressed as a percentage from the dilution curve mentioned above, can be utilized as an approximate measure for Factor VII. During therapeutic administration of Tromexan, Factor VII concentration falls by the third day to about 10%, and is kept at that level by continued administration of the drug. The prothrombin content under this therapy falls much more gradually and reaches a level of 50% only after approximately two weeks.

Russell's viper venom with lecithin has been used as a form of thromboplastin. This technique has been shown to be relatively insensitive to the changes induced by the coumarin drugs (Biggs & Macfarlane, 1949). There is some evidence (Douglas, unpublished observations, 1952) that the Russell's viper venom and lecithin form a thromboplastin that is insensitive to changes in Factor VII, and that the prolongation of the clotting time with this technique reflects only the depression of prothrombin. The antagonism of vitamin K_1 to the action of the coumarin drugs has been investigated; vitamin K_1 has been found capable of correcting or preventing the prolongation of the one-stage clotting time (Douglas & Brown, 1952; Brown & Douglas, 1952). The efficacy of vitamin K_1 in reversing the defect in thromboplastin generation and in prothrombin concentration has been recently demonstrated (Douglas, unpublished observations, 1954); administration of vitamin K_1 causes a much more rapid return to normal thromboplastin generation than occurs when the therapy is stopped without the simultaneous administration of vitamin K_1.

4. Conclusions

This paper has dealt, in the main, with the nature of the disturbance produced in the coagulation mechanism as a result of administration of the coumarin drugs. In fig. 1 it is shown how poor is the ability of the serum derived from a patient treated with Dindevan to form thromboplastin when compared with the normal. When this activity of the serum is studied daily, and expressed as a quantitative measure in comparison with the one-stage test, there is close similarity. The ability of the serum to participate in thromboplastin formation can be assayed by comparing its action with dilutions of normal serum, and thus by making an approximate quantitative measure. This is suggestive evidence that the impaired ability of coumarin serum to form thromboplastin is due to its diminished concentration of Factor VII. It is difficult, however, to design experiments to elucidate this point with absolute finality. In the thromboplastin-generation test, $Al(OH)_3$-treated normal plasma, platelets, and normal serum are incubated together to form thromboplastin. When serum from patients with Christmas disease, or from patients treated with coumarin drugs, is used in place of normal serum, there is defective thromboplastin formation. The serum from patients under therapy with the coumarin drugs frequently produces some measure of correction of the Christmas disease defect, but this is never so complete as that produced by normal serum. Koller (1954) believes that in intrinsic thromboplastin formation there is yet another coagulation component normally present in serum in addition to Factor VII, which is also reduced by coumarin therapy, and this he has called Factor X. Final clarification of these problems is still awaited. There is, however, no doubt that, whatever the final explanation may be, the serum of patients under

therapy with these drugs is very defective in its ability to form thromboplastin. It is probable that in the management of thrombo-embolic disease it is of primary importance to control the ability of the blood to form its own thromboplastin, and the real value of this group of drugs may be in this action. The interference with the extrinsic thromboplastin mechanism is probably of secondary importance.

The inability of the plasma from these patients to form thromboplastin on the addition of tissue extracts results in a prolongation of the clotting time as determined by the one-stage test of Quick. Assessment of the exact nature of this prolonged one-stage clotting time has revealed that it is caused by the deficiency of the recently recognized coagulation component—Factor VII. Factor VII is present in that fraction of plasma or serum removed by adsorption on inorganic precipitates such as aluminium hydroxide. It is not consumed during clotting, and is found, therefore, in normal serum after coagulation is complete. Thus obtained, Factor VII is free of prothrombin, Factor V and antihaemophilic globulin, all of which are used up in the process of fibrin formation. There is some measure of depression of prothrombin, but this is frequently so limited in its extent that it is unlikely to contribute significantly to the anticoagulant action of these drugs.

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CLINICAL APPLICATION OF ANTICOAGULANT DRUGS

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During the past seventeen years anticoagulant therapy has been widely used in the treatment of acute thrombo-embolism and has been adopted more recently in cases of chronic or recurring thrombo-embolic disease.

The two drugs mainly used, heparin and dicoumarol, differ completely in action as well as in chemical structure: heparin, a polysaccharide, acting directly on the clotting mechanism, takes effect almost at once in vivo, whereas dicoumarol, inactive in vitro, is thought to exert its anti-coagulant effect indirectly, by preventing the formation of prothrombin, involving a delay of about two days while the drug is absorbed and the blood prothrombin falls. In clinical use the two substances have proved to be complementary and they may be given together at the outset.

1. Drugs Acting Directly on the Clotting Mechanism

Heparin and Synthetic Heparinoid Substances

Heparin, though still costly, is the anticoagulant of choice when a short-lived immediate effect is needed. Trials of heparin substitutes have been widely reported in America and Europe, e.g. Paritol (Martin & Boles, 1952), Treburon (Hirschboeck, Madeson & Pisciotta, 1954) and Thrombocid (Aldenhoff & Werdenbach, 1952), and in Britain trials of dextran sulphate have been reported (Rickets, Walton, van Leuven, Birbeck, Brown, Kennedy & Burt, 1953).

Dosage. Heparin is standardized biologically, one international unit having the activity of $\frac{1}{130}$ mg. of standard heparin. Preparations in various strengths are on the market, the commonest being 1,000 i.u./ml., 5,000 i.u./ml. and 25,000 i.u./ml. The therapeutic range of dosage is from 5,000 i.u. to 15,000 i.u. The potency of the synthetic heparins is usually related to that of heparin.

Route of administration. By the intravenous route, heparin causes a rapid and great prolongation of the clotting time; by the intramuscular route there may be a lag of 20 minutes or more before the effect is shown. By both routes the degree and duration of the effect are proportional to the dose (Bauer, Boström, Jorpes & Kallner, 1950; Wynn, Goodwin & Birbeck, 1952), and last in the case of intravenous heparin for two to four hours, and by the intramuscular route for up to 12 hours or more.

Goodwin & McGregor (1950), in a trial of depot heparin in a Pitkin menstruum in 24 patients with thrombo-embolic disease, found that there was a latent period of six to 48 hours before an adequate effect was achieved and that intravenous heparin was required in addition if an immediate effect was desired. Local reactions were numerous and were severe in six cases. These results suggest that depot heparin has no advantages over intramuscular injection of the concentrated aqueous solution containing 25,000 i.u./ml. (Abrahams, 1950).

Administration by the subcutaneous route with hyaluronidase (Tuckman & Moolten, 1950) has not been generally adopted. The report of Litwins, Vorzimer, Sussmann, Applezweig & Etess (1951) that heparin was active by the sublingual route has not been confirmed by other workers (Tomich & Woollett, 1952; Brown & Kennedy, 1952).

Control. When heparin is administered by continuous intravenous drip or frequent intramuscular injections, the whole-blood clotting time should be estimated at least once daily, and the dosage adjusted to keep the clotting time between 15 and 20 minutes. When the intermittent method of intravenous dosage is being used, most workers agree that this is unnecessary.

Antagonist. Circulating heparin or synthetic heparinoid can be immediately antagonized by protamine sulphate (Chargaff, 1938; Jorpes, Edman & Thaning, 1939). The dose suggested by the latter is 40–50 mg. of protamine sulphate to neutralize 75 mg. of heparin (77 mg. = 10,000 i.u.), that is, 4–5 ml. of the 1% protamine sulphate solution neutralize approximately 10,000 i.u. of heparin.

Protamine sulphate itself can exert an anticoagulant effect on blood (Chargaff, 1938) and therefore, if an excess of it is present, the clotting time may increase instead of shorten.

Toxic effects. Pure heparin is non-toxic, although a few cases of individual sensitivity to it have been recorded. Jorpes (1946) reported that sensitization developed in blood donors who had been heparinized repeatedly before withdrawal of blood.

Temporary alopecia has recently been reported in patients following treatment with heparin and synthetic heparinoid drugs (Fischer, Bucher & Reich, 1953; Hirschboeck *et al.* 1954; de Takats, 1953).

2. Drugs Acting by Impeding Formation of Clotting Factors

Coumarin and Indanedione Group

For six or seven years dicoumarol was the only drug of this group available; unfortunately its effect was relatively slow to appear and to pass off (Table I). There was thus a danger of a cumulative effect unless dosage was very carefully controlled by accurate " prothrombin " time tests. During the past six years a number of substitutes have been introduced (Table I). Tromexan has been reported on in clinical trials in Great Britain; Burt, Wright & Kubik (1949) and Tulloch & Gilchrist (1951) found that it was as efficient as dicoumarol and had the

TABLE I. COUMARIN AND INDANEDIONE DRUGS IN CLINICAL USE BY THE ORAL ROUTE, SHOWING AVERAGE INITIAL AND MAINTENANCE DOSAGE, AND AVERAGE TIME TO TAKE EFFECT

Drug	Average dose (mg.)		Average time (hr.) after administration	
	Initial	Maintenance	To obtain effect	To return to normal
Dicoumarol (bishydroxycoumarin; methylene-bis-3-(4-hydroxycoumarin))	200–300	25 (or less)–100	48–72	48–120
Tromexan (ethyl biscoumacetate; bis-3-(4-hydroxycoumarinyl)-ethyl acetate)	1,200–1,500	150–900	18–28	24–72
Marcoumar (3-(1′-phenylpropyl)-4-hydroxycoumarin)	18–21	3–12	48	up to 168
Cyclocoumarol (3:4-(2′-methyl-2′-methoxy-4-phenyl) dihydropyranocoumarin)	25–75	6–12	24–72	72–168
Phenylindanedione (2-phenylindane-1:3-dione)	150–300	25–100	18–48	24–72
Dipaxin (2-(diphenylacetyl) indane-1:3-dione)	20–30	2–5	24–72	up to 144

advantage of being quicker to take effect and shorter in action because of its rapid elimination.

Toohey (1953) reported favourably on phenylindanedione. It has proved a useful substitute for dicoumarol and is considerably cheaper than Tromexan.

Warfarin sodium, a derivative of 3-(α-acetonylbenzyl)-4-hydroxycoumarin, is the only coumarin drug which has been used intravenously in a clinical trial, and it was suggested for use in cases where vomiting might interfere with oral administration of coumarin drugs. Given in doses of approximately 1 mg./kg., it brought the prothrombin level into the therapeutic range in 24 hours (Wolff, Barker, Gifford & Mann, 1953). The effect lasted for four to five days.

Control. It is necessary to control the dosage of these drugs by estimation of the prothrombin time, and in most laboratories Quick's one-stage method or a modification of it has been the method of choice.[1] A number of other methods have been suggested, and have recently been discussed by Biggs (1955). During past years several methods have been adopted of expressing the relationship between the prothrombin time of the control and the patient's plasma: (i) both results can be expressed as prothrombin time in seconds; (ii) the prothrombin index which was used by early workers, i.e.

$$\frac{\text{control prothrombin time} \times 100}{\text{patient's prothrombin time}}$$

(iii) more recently the patient's prothrombin time has been converted to a percentage of normal prothrombin activity, read from a calibration curve of the prothrombin time of serial dilutions of normal plasma.

Many workers are reverting to the first method because this eliminates any risk of error in estimating the dilution curve.

[1] See Biggs, p. 5 of this number of the Bulletin.—Ed.

The therapeutic range of prothrombin level has been defined as that at which maximum anticoagulant effect is obtained with minimum danger of haemorrhagic complications. It is usually regarded as lying between one-and-a-half and two-and-a-half times the control prothrombin time or between 10% and 30% prothrombin activity read from a plasma dilution curve. Allen, Hines, Kvale & Barker (1947) stated that their patients seldom had haemorrhage when the prothrombin level was above 10%, and seldom had recurrence or spread of thrombosis when it was under 30%.

Antagonists. It is not possible to counteract the effect of any of the coumarin or indanedione group of drugs quickly by the administration of another drug. If dangerous haemorrhage has occurred, blood transfusion is the only way of restoring the clotting mechanism quickly to normal. Vitamin K_1 administered intravenously or orally in doses of 20–200 mg. is more effective than vitamin K. It restores the prothrombin time to normal levels in eight to 12 hours (Toohey, 1952; Douglas & Brown, 1952; Chalmers, Dixon & Polack, 1954).

Toxic effects. Although dicoumarol was originally regarded as a liver poison, liver function tests have given normal results in patients who have been on coumarin drugs for several years. Haemorrhage when it occurs is due to overdosage. Agranulocytosis has recently been reported in patients who have been treated with phenylindanedione (Brown & MacMillan, 1954), and it may be that this drug and some of the other compounds with phenol rings may prove unsuitable for prolonged therapy. Fischer *et al.* (1953) reported the occurrence of alopecia in a few patients following treatment with dicoumarol and Tromexan.

3. Contra-Indications to Anticoagulant Therapy

Most workers are agreed that a haemorrhagic disorder, sub-acute bacterial endocarditis, hepatic insufficiency, renal insufficiency and the presence of ulcerative lesions, particularly in the alimentary tract, are contra-indications to the use of anticoagulant therapy. Coumarin drugs should not be used during the last three months of pregnancy (Sachs & Labate, 1949), but may be safely administered to the nursing mother as they do not appear materially to affect the prothrombin time of the suckling infant (Brambel, Hunter & Fitzpatrick, 1950).

Nutritional deficiency, particularly lack of vitamin K, or concomitant therapy with antibiotic drugs which affect the intestinal flora, or with salicylates, may increase the patient's sensitivity to coumarin drugs (Gilchrist, 1952; Owren, 1954; Glazebrook & Cookson, 1947). Sensitivity to coumarin drugs may also be increased in congestive heart failure.

Although anticoagulants are given to prevent undesirable thrombosis within vessels, their use must not interfere with haemostatic thrombosis on wounded surfaces. Normal clot formed on raw surfaces is firm and strong, but Macfarlane & Biggs (1946) have shown that excessive fibrinolytic activity may develop in blood, stress being one of the precipitating factors. In some patients, therefore, clot formed in the immediate post-operative period may be destined to lyse within 24 hours or less and, if anticoagulants are given within that period, in such cases severe haemorrhage may occur and be difficult to control, as clot formed under the influence of heparin is more tenuous and more easily disrupted than normal clot (Burt, 1954).

4. Results of Anticoagulant Therapy

a. In Venous Thrombosis and Pulmonary Embolism

The severe post-thrombotic sequels and the high incidence of fatal pulmonary embolism in cases of established venous thrombosis led early workers with anticoagulants to attack the problem in two ways: (i) by prophylactic anticoagulant treatment of all post-operative and puerperal patients, and (ii) by early diagnosis of thrombo-embolism and prompt treatment of the patients with anticoagulants.

Successful prophylactic treatment with heparin and/or dicoumarol has been reported by many early workers. Brambel *et al.* (1950), comparing the result of prophylactic anticoagulant therapy with dicoumarol therapy started 12–24 hours after delivery in 3,284 patients, found only two minor cases of superficial phlebitis in this group, whereas in the control untreated group of 3,318 patients there were 13 cases of phlebitis and three of pulmonary embolism, one of which was fatal. Post-partum haemorrhage, mainly associated with retained placental fragments, occurred in 17 of the treated group and in 12 of the control group.

In a ten-year analysis of prophylactic dicoumarol therapy in 12,834 surgical cases, Kistner & Smith (1954) found the incidence of thrombosis was 0.6% in the treated group, against 1.1% in a similar number of untreated controls; and that of fatal pulmonary emboli was 0.05% in the treated group, against 0.14% in the untreated group.

Jorpes (1947), comparing the results of conservative therapy (161 cases) and anticoagulant therapy (153 cases) in established venous thrombosis, found 42 deaths from thrombo-embolism in the first group and only one death in the second group. Matthew (1947), reporting results of anticoagulant therapy in cases of thrombo-embolism during the puerperium, found the average stay in hospital was shorter than in a similar group of conservatively treated patients. This has been confirmed by numerous other workers, including von Pugatsch (1954).

In many units the adoption of prophylactic anticoagulant therapy in all post-operative cases has not been considered justifiable, but most workers agree that it should be instituted in the post-operative period in the case of patients with a history of previous thrombo-embolic disease. On the whole, in cases of post-operative venous thrombosis in a limb, it would appear that adequate and carefully controlled anticoagulant therapy instituted early, and elevation of the affected limb (Learmonth, 1950), offer the patient the best chance of both a normal limb and protection from pulmonary emboli.

b. In the Surgery of Blood Vessels

Murray (1940) was the first worker to report the use of heparin to prevent thrombosis at the suture line after operations on blood vessels, and he indicated that this could be done by regional administration as well as by systemic administration (Murray, Jaques, Perrett & Best, 1937). Later workers are agreed that prophylactic anticoagulant therapy is not necessary in the case of operations on blood vessels where the flow of blood is rapid and conditions allow accurate approximation of the intima (Learmonth, 1951). However, Learmonth points out that, where there is doubt about the smoothness of the suture line, the danger of thrombosis is imminent. Immediate heparinization is indicated and should be continued for 48 hours. This type of therapy is also likely to be needed following such operations as " désoblitération " of thrombosed arteriosclerotic vessels when a rough internal surface may be left, and Kinmonth (1951) suggests that some form of regional heparinization may prove safer in these cases than systemic dosage. Drugs of the coumarin group, with their slow initial effect, have no place alone in the immediate post-operative period, but may be used in combination with heparin.

c. In Chronic Obliterative Arterial Disease[2]

Examination of the amputated limbs of patients with gangrene caused by obliterative arterial disease suggests that in many cases the final worsening has been due to recent spread of intra-arterial thrombosis. Theoretically, therefore, the exhibition of anticoagulants should prevent such spread and thus preserve the patency of collateral vessels on which the nutrition of the limb depends. This type of therapy does not offer any prospect of dramatic improvement, but it may possibly facilitate recanalization of recently thrombosed vessels in man, as has been shown in animals (Kubik & Wright, 1951; Wright, Kubik & Hayden, 1953). Allen *et al.* (1947) reported that, after prolonged anticoagulant treatment of 38 patients suffering from occlusive arterial disease, they were unable to come to any conclusion relative to the effectiveness of this type of treatment. Burt (1955) has reported the use of long-term anticoagulant therapy in 51 patients with severe obliterative arterial disease. Only 15 had returned to work for periods of up to two-and-a-half years.

Recently, Engelberg & Massell (1953) have suggested what is possibly a more fundamental attack on the problem of arteriosclerotic vessels—that through the chylolytic action of heparin on lipoproteins the greasy coating of vessel walls may be diminished and oxygen exchange facilitated, thus aiding tissue nutrition. The dosage used, 100 mg. of heparin intravenously twice weekly, appears to be insufficient to cause more than a brief effect on lipoproteins.

d. In Acute Peripheral Arterial Block by Thrombosis or Embolism

In cases of sudden arterial occlusion—embolic or thrombotic—anticoagulants, particularly heparin, offer a means of preventing consecutive thromboses and thus give time for a collateral circulation to develop and for the surgeon to decide whether it will be necessary to operate. Indeed, some surgeons believe that in all such cases conservative therapy should be adopted in the first instance. Learmonth (1950) states that intravenous heparin (12,500 i.u.) and morphia, if necessary, should be given at once, and Tromexan therapy started. The only exceptions he would consider to this method of treatment are when the embolus has lodged at the aortic bifurcation, or if an embolus had previously lodged in the same limb. He reported that, of 22 patients with sudden arterial occlusion treated with anticoagulants from the first, 12 showed immediate improvement, six required exploration or embolectomy, and two patients in whom treatment was started late required amputation. Two patients subsequently died of further multiple emboli. Learmonth also mentions the rapid relief of pain by heparin in cases of arterial embolus, possibly through a vasodilator effect. Abrahams & Howarth (1950) reported a rise in forearm blood flow following injection with heparin.

e. In Coronary Thrombosis

In 1946, Nichol & Page, Peters, Guyther & Brambel, and Wright published papers which indicated a striking reduction

[2] See also Learmonth & Slessor, *Brit. med. Bull.* 1952, 8, 375.—Ed.

in the mortality rate and incidence of thrombo-embolic complications in patients treated with anticoagulants during the first few weeks after acute coronary thrombosis. Since then a large number of workers have reported similar findings, and Tulloch & Gilchrist (1951) have analysed the reports of treated and control cases in the literature. In a total of 1,833 cases of acute myocardial infarction, 883 were treated with heparin and/or dicoumarol, and 950 were treated by conservative measures. Of the 883 patients treated with anticoagulants, 143 died (16.2%) and there were 78 thrombo-embolic episodes (9%), while in the control group 291 (30.6%) died and there were 207 (21.8%) thrombo-embolic episodes. It is unlikely that chance determined that death and thrombo-embolic episodes should both be halved in treated patients in comparison with untreated patients. Somerville (1954) quotes Parkinson & Bedford (1928), Blumer (1937) and Hellerstein & Martin (1947) as giving 8%, 13.9% and 11.5% respectively as the incidence of embolic incidents in their series treated without anticoagulants, and he points out that these figures are similar to those in the group of patients who had received anticoagulants reported by Gilchrist (1952). He suggests the paradox is explicable by the lack of agreement among workers as to what constitutes thrombo-embolism, but, in the series reported by Tulloch & Gilchrist (1950), the control and treated patients were observed by the same workers, who would presumably be applying the same diagnostic criteria to both groups.

Duration of therapy. Wright, Beck & Marple (1954a) considered that four weeks should be the minimum duration because thrombo-embolic complications and deaths were more frequent during the fourth week of the illness in the control group than in the treated group in their series.

Shock. Gilchrist (1952) and Loudon, Pease & Cooke (1953) recorded noticeable improvement in severely shocked patients following the institution of anticoagulant therapy, and found that the incidence of deaths in shocked patients was lower in those who had received anticoagulants than in the control group.

Haemorrhage. The danger of haemorrhagic complications has been put forward as a contra-indication to anticoagulant therapy in cases of myocardial infarction. Tulloch & Gilchrist (1950) and Loudon *et al.* (1953) do not report any severe haemorrhage in their series of 70 and 75 treated patients respectively, but Wright *et al.* (1954a) state the clinical incidence of bleeding to have been 13% in the treated group of 589 cases, and 5% in the 442 control cases. In the necropsy material, the incidence of macroscopic evidence of intracardiac haemorrhage was 52% in the controls and 66% in the patients treated with anticoagulants, and the incidence of microscopic haemorrhage was 11% and 20% respectively. No increase in sub-intimal haemorrhage was found in the coronary arteries.

f. In Angina Pectoris

A number of workers have reported symptomatic improvement in cases of angina pectoris following heparin treatment in doses of 50–100 mg. once or twice weekly. Brown & Rykert (1954), however, quoting the results in 88 previously reported cases and adding 20 of their own, found, when using a double blind control method, that a placebo was as effective as heparin in preventing attacks of anginal pain. They concluded that in cases of angina pectoris the psychological effect of any therapy was striking and that it seems unlikely that bi-weekly injections of heparin are of more value than the knowledge that some form of treatment is being administered. However, in a group of 10 patients suffering from angina decubitus, Wood (1949) obtained better results by the use of continuous anticoagulant therapy with heparin and dicoumarol than he did in the case of nine similar patients treated by conservative methods.

Smith & Papp (1951), having found that 30 out of 200 patients with myocardial infarction had had the prodromal syndrome of increasing anginal pain, thereafter treated with anticoagulants 14 patients who developed this syndrome. In six, infarction did not develop, but the remaining eight developed signs of myocardial infarction and three of this group died. At necropsy, advanced coronary sclerosis was found in two of the patients who died, and the authors suggest that the failure of prophylactic anticoagulant therapy in other cases may be similarly explained.

g. In Miscellaneous Conditions

The use of heparin in the hope of preventing intravascular thrombosis and local fibrin deposition in the neighbourhood of inflammatory foci, and thus facilitating access of antibiotics and promoting healing, has been reported (Sandblom, Ekström & Quist, 1948). Stoker (1951) reported good results in the healing of burns following the use of heparin–saline compresses. He considered that heparin acted locally as a combined antibacterial and decongestive agent. In neither of the above conditions has the use of heparin been generally adopted.

Keller (1955) has reported lowering of blood pressure in patients with hypertension, following treatment with 5,000 i.u. of heparin thrice weekly.

5. Long-Term Anticoagulant Therapy in Out-Patients

In the early years of dicoumarol treatment, out-patient therapy fell into disrepute because of fatal haemorrhage, which occurred in a number of patients who took, or were given, this drug without laboratory control of dosage. Many workers considered that treatment of the ambulant patient with dicoumarol was unjustified in view of the risk of haemorrhage, even when frequent prothrombin estimations were made. Scott & Lissimore (1944) record what is probably the earliest instance of long-term anticoagulant therapy in Britain. A man of 62 years suffering from lymphatic leukaemia, who for two years had had recurrent attacks of mesenteric thrombosis, proven at laparotomy on one occasion, was put on anticoagulant therapy with dicoumarol. In a later paper Scott (1952) reported that this man remained free of attacks of abdominal pain for the remaining three years of his life, except on one occasion when the prothrombin level had risen to 85% of normal. Prothrombin tests, carried out at weekly or fortnightly intervals at first, were done at monthly intervals during the last two years of treatment. Following this experience, Scott gave dicoumarol to 40 out-patients, including 25 cases of coronary thrombosis, 11 of cerebral thrombosis and four cases of mesenteric thrombosis, for periods of six months to three years. Only two patients in this series had further thrombo-embolic complications while on dicoumarol therapy. No haemorrhages occurred.

Meanwhile American workers, having noted the diminution in number of thrombo-embolic episodes in cases of acute myocardial infarction treated with anticoagulants, instituted

long-term prophylactic anticoagulant therapy in chronic thrombo-embolic disease such as the following: recurrent myocardial infarction, multiple embolic episodes, recurrent thrombophlebitis, idiopathic or recurrent pulmonary embolism or thrombosis. Lund (1953) collected from the literature reports of 225 patients treated as out-patients for periods of from one month to six years. Since then a number of other reports have appeared—Wright *et al.* (1954b), Tulloch & Wright (1954), Stephens (1954), Owren (1954) and Burt (1955). If that of Scott (1952) is included and allowance made for duplication of reports on the same patients by taking only the latest report of each author, a total of 1,003 patients has been reported as having been treated on an out-patient basis for periods of from one month to eight years. Dicoumarol was used in the majority of patients, but Tromexan, phenylindanedione and cyclocoumarol have also been used. Most workers preferred to use a scheme of continuous daily dosage in order to keep the prothrombin activity as far as possible between 10% and 30% of normal. Scott (1952) and Burt (1955) used mainly intermittent dosage and did not depress prothrombin to such low levels, aiming at ranges of 50% to 70% and 30% to 50% respectively. However, as estimations of the prothrombin level differ markedly with different types and potencies of thromboplastin, it is not possible to ascertain whether the prothrombin levels in the different groups are comparable or not.

Results. It was recognized by all the workers in this group that statistical evidence for or against the value of this type of therapy would be difficult to obtain because spontaneous remissions in thrombo-embolic disease may last for years, but there was general agreement that while these patients were on anticoagulant therapy the incidence of thrombo-embolic episodes was less than it had been before. Tulloch & Wright (1954) reported that, in 227 patients who had had 546 thrombo-embolic episodes before anticoagulant therapy was instituted, only 40 episodes occurred during anticoagulant therapy.

Complications. Haemorrhage was the most serious complication which occurred, and it was reported as severe in 46 (4.6%) of the 1,003 patients and was fatal in six (0.6%) of these.

With increasing experience in the management of this type of therapy, the incidence of haemorrhage should be considerably diminished, but it is always a danger in patients with hypertension and, in these, long-term anticoagulant treatment should be given with caution. In the future, anticoagulant therapy may have a large part to play in the treatment of chronic thrombo-embolic disease in ambulant patients. Owren (1954) reports that he now has 700 patients in whom anticoagulant therapy is controlled from his clinic. In many cases, blood for testing is sent by post, and he considers that his "prothrombin proconvertin" test is particularly suitable for this type of work.

All the workers have emphasized that this type of therapy should never be used without strict laboratory control of prothrombin level and close co-operation between patient and doctor.

6. Comments

The decision to give or withhold anticoagulant drugs is a matter for the individual clinician, but there is an accumulating mass of evidence that, carefully used, these drugs will materially diminish the morbidity in cases of deep venous thrombosis, acute arterial occlusion and pulmonary embolism, diminish the death-rate from myocardial infarction and lower the incidence of thrombo-embolic incidents. There is also evidence that, used on a long-term basis in cases of recurrent myocardial infarction, of auricular fibrillation with previous embolic episodes, and of recurrent idiopathic venous thrombosis and migratory phlebitis, the number of attacks can be diminished or prevented. It is not yet certain whether long-term anticoagulant therapy will materially affect the prognosis in cases of chronic obliterative arterial disease. The coumarin and indanedione drugs should never be given without laboratory control.

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THE NATURE OF THE FIBRINOGEN–THROMBIN REACTION

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1 Historical
2 The nature of fibrinogen
3 The action of thrombin on fibrinogen
4 The fibrin clot
References

The present contribution is no more than a brief survey of this very large subject, and the main emphasis will be given to the recent work on the chemical events in the conversion of fibrinogen to fibrin.

1. Historical

It is now almost 100 years since Denis (1859) showed that coagulable fibrinogen could be separated from the other proteins of plasma, and it is perhaps rather surprising that only in recent years has unequivocal evidence been obtained as to the nature of the action of thrombin in the clotting process. In vindication of the views held by early investigators (Heubner, 1903; J. Mellanby, 1909), thrombin does now appear to be a rather specific protease, though it must be admitted that the idea of the proteolytic nature of the clotting process arose at first from erroneous evidence. The fibrinogen preparations of those days were often contaminated with other globulins which remained in the clot liquor, giving rise to the view that fibrinogen itself was split by thrombin into insoluble fibrin and a soluble globulin. This view persisted in spite of Huiskamp's demonstration in 1905 that a fibrinogen could be prepared which did not yield a soluble protein on clotting.

The concept of proteolysis was resuscitated much later by other misleading experiments and analogies, having their origin in the fact that thrombin preparations were often contaminated with one or more plasma proteases (plasmin). Clots allowed to stand would undergo fibrinolysis, precisely like those formed with accredited proteolytic enzymes such as papain (Eagle & Harris, 1937) and some snake venoms (Eagle, 1937). Soon, however, it was to be shown by Hudemann (1940) and Seegers (1940) that lysis is not an intrinsic part of thrombin action, and, by using purified enzyme and substrate, some workers recovered most of the fibrinogen as insoluble fibrin—Hudemann (1940), 98%; Ware, Guest & Seegers (1946), 95–101.4%. Such results naturally gave support to theories which regarded thrombin as a denaturase (Wöhlisch & Jühling, 1938; Wöhlisch, 1940, 1949), or as a coacervase (Mommaerts, 1945); so much so that Astrup, reviewing the situation in 1950, could say, " The hypothesis that thrombin is a proteolytic enzyme is now regarded as obsolete, as all the nitrogen in purified fibrinogen may be recovered as fibrin ". At that juncture, however, it was shown unequivocally (Bailey, Bettelheim, Lorand & Middlebrook, 1951) that by the action of plasmin-free thrombin certain *N*-terminal amino acids present in fibrinogen disappeared and were replaced by new *N*-terminal groups in the peptide chains composing fibrin; in other words, it seemed likely that thrombin was a rather specific protease, breaking a few peptide links and liberating non-protein nitrogen into the clot liquor. It must be pointed out, however, that although the peptide bond is the predominating linkage in proteins, it has not yet been proved in this case that the liberation of carboxyl groups accompanies the liberation of amino groups. These chemical events are considered more fully later.

2. The Nature of Fibrinogen

Fibrinogen is a globulin with an acid isoelectric point of pH 5.5 (Mihalyi, 1950a), soluble in salt solutions of ionic

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TABLE I. CHARACTERISTICS OF FIBRINOGEN

Properties	Human fibrinogen	Reference	Bovine fibrinogen	Reference
N content (%)	17.1	Bailey (1944)	—	—
N-terminal amino acids	Tyrosine, alanine	Bailey (unpublished) Lorand & Middlebrook (1953)	Tyrosine, glutamic acid	Bailey, Bettelheim, Lorand & Middlebrook (1951)
Sedimentation constant	8.5–9 7.63	Holmberg (1944) Caspary & Kekwick (1954)	8.4–8.6	Koenig & Pedersen (1950)
Intrinsic viscosity	0.25	Oncley, Scatchard & Brown (1947)	0.25	Nanninga (1946)
Rotary diffusion constant ($sec.^{-1}$)	35,000	Edsall, Foster & Sheinberg (1947)	39,400	Hocking, Laskowski & Sheraga (1952)
Molecular weight ($\times 10^{-3}$):				
osmotic pressure	580	Oncley *et al.* (1947)	440	Nanninga (1946)
light-scattering	440	Morrison & Lontie (unpublished)	540 407 340	Steiner & Laki (1951) Hocking *et al.* (1952) Katz, Gutfreund, Shulman & Ferry (1952)
sedimentation-diffusion	341	Caspary & Kekwick (1954)	—	—
Molecular length (Å):				
double refraction of flow	700	Edsall *et al.* (1947)	670	Hocking *et al.* (1952)
osmotic pressure and viscosity	—	—	725	Nanninga (1946)
sedimentation and viscosity	700	Oncley *et al.* (1947)	—	—
light-scattering	—	—	840	Steiner & Laki (1951)
Molecular diameter (Å)	38	Oncley *et al.* (1947)	38	Oncley *et al.* (1947)

strength 0.3 at neutral pH. It is the first protein to be precipitated from plasma by salt (15–20% saturated ammonium sulphate), or by ethanol (10% (v/v) at pH 7).

Estimates of its size have been rather conflicting, and in recent years have shown a downward trend. It does appear, however, that human and bovine fibrinogen have similar constants, and some of these are given in Table I. From Edsall's critical discussion of the data (Edsall, 1953), it seems likely that the most acceptable value of the particle weight is near 350,000 and, since then, a careful ultracentrifuge study by Caspary & Kekwick (1954) has defined the particle weight of human fibrinogen as 341,000. At very low protein concentrations there was evidence for dissociation into sub-units of particle weight 130,000.

Viscosity and flow birefringence studies show that fibrinogen is an asymmetrical molecule and, on the assumption that it resembles an ellipsoid of revolution, its length, calculated from both birefringence and light-scattering, is about 700 Å and its width 38 Å (Table I). Hall (1949) has reported that, in the electron microscope, the most commonly occurring structures are rod-shaped, of about the length deduced above, but other electron microscope studies have given very conflicting results.

Both fibrinogen and fibrin contain carbohydrate corresponding to 3% of reducing sugar (mannose and galactose) and 0.6% of hexosamine (Consden & Stanier, 1952). Like the muscle proteins, myosin and tropomyosin, fibrinogen contains a high proportion of hydrophilic side chains (56% of the total groups), and a particularly large proportion of aliphatic hydroxy acids (14%) (see Tristram, 1949). Its wide-angle *x*-ray pattern is predominantly that of the α-keratin-type proteins, but both it and fibrin show some admixture with β-keratin-type chains (Bailey, Astbury & Rudall, 1943). It is not known whether the β-keratin parts of the fibrinogen and fibrin chains arise from transformation of α-keratin-type chains during manipulation, or whether they are an intrinsic feature. For reasons given in section 4, this point has some bearing on hypothetical mechanisms associated with the aggregation of fibrin particles.

3. The Action of Thrombin on Fibrinogen

The clue to the elucidation of the chemical mechanism of clotting came from the demonstration that the *N*-terminal groups of bovine fibrinogen are tyrosine and glutamic acid, whereas those of fibrin are tyrosine and glycine. The limited accuracy of the analyses, and the uncertainty surrounding the particle weight of fibrinogen, leave doubt as to the number of these end-groups per molecule. The analytical figures, in grams of protein containing one mole, were 150,000 for tyrosine, 265,000 for glutamic acid, and 125,000 for glycine, according to Bettelheim & Bailey (1952); the corresponding estimates of Lorand & Middlebrook (1952) were 225,000, 500,000 and 100,000. Perhaps the truth lies between the two sets of figures, giving for a particle of 350,000 two *N*-terminal tyrosine chains, one glutamic chain, and, in the case of fibrin, the substitution of the latter by 2–3 glycine chains. The important point is the stoicheiometrical relation of glutamic and glycine end-groups. From the clot liquor, Lorand (1952) isolated peptide material which he considered to be homogeneous, but, in our own independent investigation,

two peptides were found, readily separable by electrophoresis at pH 4.1 (Bettelheim & Bailey, 1952), or, as their dinitrophenyl-derivatives, by adsorption and differential elution from talc. One of these peptides, A, carries the *N*-terminal glutamic acid lost from fibrinogen, whilst the other peptide, B, appears not to have an *N*-terminal amino acid when tested by Sanger's fluorodinitrobenzene method. The *N*-terminal glycine residues of fibrin represent the "scars" left behind on the main body of the protein molecule when the peptides are split off. In human fibrinogen, *N*-terminal alanine replaces glutamic acid (Lorand & Middlebrook, 1953), but otherwise the general picture, including that of the peptides released (Bailey, unpublished, 1954), appears to be similar to that described above.

Both peptides A and B are of rather complex constitution, containing a variety of amino acids and having minimal molecular weights of about 3,000. Both are markedly acidic, with isoelectric points near pH 3.2. Peptide B contains tyrosine with the phenolic hydroxyl group conjugated with sulphuric acid (Bettelheim, 1954)—a unique finding, as yet, for a protein, whose functional significance cannot be assessed at present. Neither peptide has marked anticoagulant properties.

From the above facts, it appears that thrombin attacks fibrinogen at bonds involving the amino groups of glycine. Information as to other participants in the susceptible bond has come from the work of Sherry & Troll (1954), who have shown that plasmin-free thrombin attacks synthetic compounds at bonds involving the carboxyl group of arginine; for instance, tosyl arginine methyl ester is split into tosyl arginine and methanol. Analogous compounds containing amino acids other than arginine were not attacked. There is thus evidence, albeit indirect, that thrombin splits arginyl glycine links in fibrinogen, though it is disappointing to find that it does not attack the glutamyl-arginyl-glycylphenylalanine sequence of the B chain of insulin (Bailey, unpublished work). The ability of thrombin to split ester bonds is not to be taken as evidence for the occurrence of such bonds in fibrinogen, since many proteolytic enzymes exhibit esterase activity (Neurath & Schwert, 1950). Tosyl arginine methyl ester delays the clotting of fibrinogen by what appears to be a competitive inhibition.

Thrombin is unique among the known proteolytic enzymes in the narrow specificity of its action; only one peptide bond in about 1,000 is split during clotting. The plasmins, on the other hand, have a related but wider specificity, and, like trypsin, attack both arginine and lysine substrates (Troll, Sherry & Wachman, 1954; Troll, 1954). The amino-acid sequence from the *N*-terminal ends of the glycyl chains of fibrin and from the carboxyl ends of the A and B peptides may give the final answer to the specificity of thrombin, and show whether the substrate studied by Sherry and Troll has given definitive evidence of its action on fibrinogen or whether it possesses an element of factitiousness.

4. The Fibrin Clot

The fibrin gel is essentially a three-dimensional network of protein strands in the interstices of which large amounts of liquid can be held (see Ferry, 1948). Under favourable conditions the slightest tension or pressure causes extrusion of liquid as the strands aggregate by sideways cohesion. For example, if a clot is formed in a long, narrow tube and then pulled gently at one end, the tension suffices to synaerize the clot, and a fine silken thread is obtained on which the expressed liquid hangs like dew on a spider's web.

The ultimate particle from which these aggregates form must, from the chemical evidence alone, be very like the original fibrinogen, which has lost only 3% of its protein nitrogen (Lorand, 1952). But physical evidence also suggests that the fibrin monomer, as we might call it, is indeed like fibrinogen in size and shape. Thus, in the presence of high concentrations of urea, which prevent aggregation and favour disaggregation, fibrinogen and fibrin have the same viscosity (Mihalyi, 1950b) and the same sedimentation constant (Ehrlich, Shulman & Ferry, 1952). Moreover, Laki (1951) has shown that at pH 5 thrombin alters fibrinogen so that it clots readily at a higher pH, though in acid solution no clotting occurs and the particles have the same sedimentation properties and viscosity as those of fibrinogen. Bettelheim (1953) has confirmed that the chemical events under acid conditions are in no way different from those at a higher pH.

There is now a large literature dealing with the effects of ions, pH and several types of organic compounds on the formation and nature of the clot. Clotting is retarded by high concentrations of salt (Mommaerts, 1945) and is suppressed not only by a too acid pH but also by one too alkaline (pH above 10) (Edsall & Lever, 1951; Shulman & Ferry, 1950). Alkali favours the formation of transparent, friable gels, difficult to synaerize, and these consist of a mesh-work of fine strands. At pH 7, however, the clot is coarse and opaque and retracts easily, owing to extensive lateral aggregation into thick fibres (Ferry & Morrison, 1947). The adsorption of anions such as iodides and thiocyanates (Edsall & Lever, 1951) favours the formation of a transparent clot, and it is clear that an over-all change in net charge, whether by this means or by altering pH, affects clotting by simple electrostatic effects. By diminishing electrostatic interactions, high salt concentrations probably act in a similar manner.

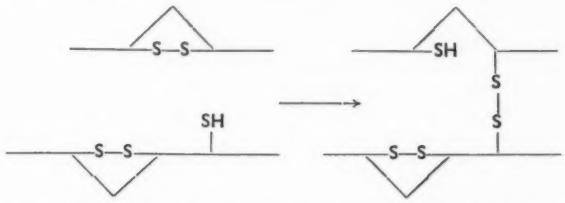
In the fibrin monomer, there must be definite local sites—possibly near the *N*-terminal glycine residues—which initiate aggregation, and although it may be that electrostatic effects are involved here, it seems probable that hydrogen bonding plays a very important role. Reagents such as urea and hexamethylene glycol, which compete for hydrogen bonds, retard or inhibit clotting (Edsall & Lever, 1951; Ferry & Shulman, 1949), and in these systems the type of product present is a function both of inhibitor and protein concentration; the fibrin monomer is present and its formation is particularly favoured by dilution (Ehrlich *et al.* 1952). In addition, there can be seen in the ultracentrifuge a very sharp peak which probably comprises intermediates of varying length but about twice the width of the monomer (Shulman & Ferry, 1951; Ehrlich *et al.* 1952).

The nature of the groups responsible for forming the intermolecular hydrogen bonds which hold the fibrin monomers together in the gel network is not known. They might belong to amino-acid side-chains arranged in a specific spatial pattern, or they might be segments of peptide chains present in the extended or β-configuration and thus able to form bonds between the —CO—NH— groups of their backbones—the "backbone association" by which sheets of polypeptide chains are built up in the model for β-keratin (Astbury & Woods, 1933). The latter possibility is an attractive one. The significance of the β-configuration lies in the fact that the hydrogen bonds are available for linking one molecule to the

next, making the protein insoluble; in the folded α-configuration, whatever its exact nature, there is general agreement that hydrogen bonds are satisfied within the chain, holding the fold in place, so that they do not prevent the protein molecules from separating and dissolving. Only a small proportion of fibrin would need to be in the β-configuration to account for gel formation; if the β regions were localized at or near the ends of the elongated monomer particle, aggregation would be predominantly end to end. It is not inconceivable that thrombin might cause a local $\alpha \rightarrow \beta$ transformation, by analogy with the way in which some proteolytic enzymes initially denature protein substrates (Linderstrøm-Lang, 1949). Or it might be supposed that the local amino-acid composition favours the β-configuration by the absence of bulky side-chain residues, as in silk fibroin, the insolubility of which appears to be caused by β regions, which account for a high proportion of the total, and contain only the small residues of glycine, alanine and serine (Drucker & Smith, 1950; Ambrose, Bamford, Elliott & Hanby, 1951).

Electrostatic forces probably play a subsidiary role in clot formation—perhaps in orienting the molecules in positions suitable for association (Ferry, Katz & Tinoco, 1954). Since the peptides split off from fibrinogen are so acidic, their removal must cause an appreciable alteration in the electrostatic pattern of the molecule.

An interesting property of fibrin clots remains to be mentioned. Clots formed from pure fibrinogen are quite soluble in urea, acid and alkali, but those formed in the presence of calcium ion plus serum are not (Robbins, 1944; Lorand, 1948). The presence of an SH-combining reagent such as iodoacetamide in addition to calcium ion and serum factor produces the urea-soluble clot (Loewy, private communication, 1954), suggesting that loss of solubility is associated with S—S cross-linking, which under the latter conditions is prevented by combination of SH with thiol reagent. Perhaps the disulphide exchange mechanism suggested by Huggins, Tapley & Jensen (1951) is operative here, whereby *intra*chain S—S bonds are transformed into *inter*chain bridges:



Whether the process has any physiological importance is not immediately obvious.

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LARGE-SCALE SEPARATION OF CLOTTING FACTORS FROM HUMAN PLASMA

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During the last ten years, intensification of research on the problems of blood coagulation has revealed an ever-growing complexity of the mechanisms involved, concomitant with the demonstration of a plethora of hitherto unrecognized participating components.

In a system so delicately balanced it is not surprising to discover that many of the components are very labile. This inherent lability is in some instances apparently enhanced by the presence in blood of substances which, by neutralizing the effects of participants in the forward reaction, help to maintain the normal fluid equilibrium. In comparison therefore with other constituents of plasma, such as, for example, albumin and γ-globulin, the separation and purification of non-cellular components of the clotting mechanism frequently present unusual difficulties.

With the advance of knowledge, the value of providing components of the clotting system in purified and concentrated form suitable for clinical use has become apparent, and relatively large quantities are required. For the scale of operation used in research it is justifiable, and usually simpler, to develop a method which, for the isolation and purification of one of the components of the clotting mechanism, involves jettisoning the remainder of the plasma proteins, in order to be able to study the properties and function of this particular component. The fractionation of so valuable a commodity as human plasma by such methods on a large scale is indefensible and impracticable. The isolation of clotting factors has necessarily to be fitted into a logical scheme of fractionation, by means of which as many as possible of the constituents of human plasma are recoverable in reasonable yield. It is, of course, almost unnecessary to add that the final products must be bacteriologically sterile, and indeed it is considered highly desirable that the fractionation procedures should be carried through with aseptic precautions. In view of these considerations it will be recognized that there are extensive disciplinary restrictions within which a fractionation scheme providing clotting factors from human plasma must be designed.

Animal plasmas, for example bovine plasma, may be and have been utilized to provide clotting factors, especially thrombin. Such materials are more suitable for in-vitro use than for parenteral administration into man, since, being heterologous, they are potentially antigenic. Before they might safely be used parenterally it would be necessary either to establish their non-antigenicity, or else to despeciate them in such a manner that their specific biological characteristic remained unaltered—a rather difficult task with labile substances.

The clotting factors that have so far been isolated successfully on a large scale from human plasma are fibrinogen, thrombin and prothrombin. The fibrinogen preparations usually contain antihaemophilic globulin (AHG), though the recovery of this substance has been rather poor and variable in general experience.

Fractionation Methods

The reintroduction by E. J. Cohn and his colleagues of the use of water-miscible solvents—such as ethanol, methanol, acetone and ethyl ether—for the precipitation of proteins provided systems with an increased number of variables for exploitation in the separation of protein components from complex mixtures. In particular, the employment of these solvents eliminated what had previously been a necessity—the use of neutral salt concentrations of the order of 2–3 M to precipitate the more soluble proteins such as albumin.

The variables available are solvent concentration, total protein concentration, pH, ionic strength (a function of salt concentration) and temperature. The last-mentioned variable is effectively restricted to the range 0° to −10° C.; at higher temperatures solvents tend to cause irreversible changes in the solubility of proteins and loss in biological activity.

A further advantage of solvents is that the precipitating agent may be removed by freeze-drying instead of by the tedious dialysis techniques required by neutral salt fractionating procedures. Moreover, the use of solvents enables an aseptic fractionation technology to be readily developed; the asepsis is aided by the bacteriostatic properties possessed by most of the solvents that may be utilized, and by the low temperatures at which the procedure is carried out.

More recently, the possibilities of qualitative alteration of the ionic environment by the addition of minimal amounts of heavy metal salts have been explored. To remove such heavy metals from the final products, solutions are passed through columns of suitable ion-exchange resins.

Ethanol Fractionation

In the earlier methods developed by E. J. Cohn and his colleagues, citrated human plasma was fractionated in ethanol-containing systems, and several procedures differing in detail were evolved. In one of these (Method 6; Cohn, Strong, Hughes, Mulford, Ashworth, Melin & Taylor, 1946), a fibrinogen-containing fraction (Fraction I) was separated at pH 7.2 with 8% (v/v) ethanol at −3° C. This was effectively a concentrate of which about 60% of the protein was fibrinogen, the remainder being mostly plasma protein occluded in the precipitate. It also included a high proportion of the antihaemophilic factor. By adjustment of the supernatant solution to pH 6.8, reduction of the ionic strength to 0.09 and increase of the ethanol concentration to 25% (v/v) at −5° C., Fraction II + III was precipitated, containing the bulk of the β- and γ-globulins including most of the prothrombin. Subfractionation of Fraction II + III (Oncley, Melin, Richert, Cameron & Grose, 1949) provided prothrombin, as well as other products; this could be converted to thrombin, if desired, with human placental thromboplastin under optimal conditions of pH and calcium ion concentration. Further main fractions concentrated preferentially the α-globulins (Fractions IV—1 and IV—4) and albumins (Fraction V), so accounting for the greater proportion of the main plasma protein constituents.

More recently, blood has been rendered incoagulable by passing it through an ion-exchange resin column to remove calcium. If suitable amounts of barium sulphate are added to the plasma separated from such blood (Surgenor, Alexander, Goldstein & Schmidt, 1951), the prothrombin and the precursor of Factor VII are adsorbed, and can be eluted subsequently with dilute sodium citrate solutions. The prothrombin obtained by this method is of higher purity than preparations obtained by previous procedures, and it would appear not to be convertible to thrombin by the addition of thromboplastin and calcium only. From the adsorbed plasma a fibrinogen fraction, similar to Fraction I above, may be separated by the addition of 6% (v/v) ethanol at pH 6.8 and −2° C.; 60% of the total protein of this fraction is clottable. It also contains most of the antihaemophilic globulin and plasminogen.

In Fraction II + III, subsequently separated, is found all of the Factor V activity.

The further purification of fibrinogen has been described by Morrison, Edsall & Miller (1948). They obtained products varying from 95% to 98% in their content of clottable protein but showing two components in the ultracentrifuge; no data relating to freedom from plasminogen and plasmin, which are difficult to separate from fibrinogen, were provided.

Ethyl Ether Fractionation

A fractionation scheme making use of ethyl ether as a precipitating agent, and in which aseptic procedures are utilized throughout, has been in operation for some time in Great Britain (Kekwick, Mackay & Record, 1946; Kekwick & Mackay, 1954). By the addition of 11% (v/v) ether to human citrate plasma at 0° C., a fibrinogen-rich fraction may be separated. This contains 90% of the initial plasma fibrinogen, and 50% of the total protein appears in the clot formed on the addition of thrombin. By suspending the crude precipitate in a citrate–saline solution containing 8% (v/v) ether at 0° C., and then by centrifuging down the washed precipitate, occluded proteins are removed and a preparation of which 80% of the total protein is fibrinogen is obtained. This is free from prothrombin, but the antihaemophilic globulin which separates with the first crude precipitate is retained in the washed fibrinogen, which in addition contains some plasmin and plasminogen. Further fractionation of this material has provided fibrinogen preparations, free from plasminogen and plasmin, of which more than 97% of the total protein nitrogen is recovered in the clot formed with thrombin (Kekwick, Mackay, Nance & Record, to be published). These preparations show only one component in the ultracentrifuge (Caspary & Kekwick, 1954).

The supernatant solution from the fibrinogen is next adjusted to pH 5.3, while the ether content is simultaneously reduced to 10% (v/v) at 0° C.; this causes the precipitation of a prothrombin-rich fraction. Occluded protein is removed from this precipitate by suspending the precipitate in distilled water at 0°–2° C. and centrifuging down. The washed precipitate may be directly converted to thrombin, under optimal conditions, with calcium and human placental thromboplastin, when a preparation of sufficient purity for clinical purposes is obtained. Alternatively, the washed precipitate, which also contains the bulk of the plasma plasminogen, may be subfractionated (for example, by adsorption with barium sulphate), to provide highly purified preparations of prothrombin.

Subsequent steps in the main fractionation lead to the recovery of γ-globulin and albumin from successive plasma supernatant residues.

Conclusion

It would seem that, for direct clinical use, the degree of purity required for preparations of the clotting factors need not be of a very high order. It is of course necessary that the purity should be such that an adequate dose may be contained in a volume small enough for convenient administration.

However, the diagnosis of defects of the clotting mechanism frequently involves the provision of clotting factors of high purity. So far, fibrinogen, prothrombin and thrombin have been prepared on a large scale in a condition which may be described as approaching biochemical purity. As our knowledge of the characteristics of other clotting factors increases, it will undoubtedly be feasible to separate them on a sufficiently large scale as sub-fractions from a major fractionating system, with the degree of purity essential for diagnostic and research procedures.

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CHEMISTRY OF COUMARIN ANTICOAGULANT DRUGS

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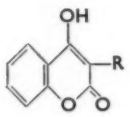
1 Classification of coumarin anticoagulants
2 Relationship between chemical structure and anticoagulant activity
3 Vitamin K and anticoagulant action
4 Distribution and metabolism of coumarin derivatives in the body
5 Discussion
References

Coumarin anticoagulant drugs are relatively simple chemical compounds which have a profound effect on blood coagulation when administered to animals or man. Though they are extensively used clinically and have been proved to produce therapeutic benefit in diseases associated with intravascular clotting, the chemical processes involved are by no means fully understood.

1. Classification of Coumarin Anticoagulants

The coumarin anticoagulant drugs in use at present may be divided into four main groups according to their chemical structures.

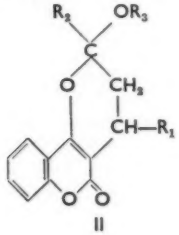
i. 3-Substituted-4-hydroxycoumarin derivatives represented by the general formula (I) in which R is an aryl or aralkyl group.



I

Notable examples of this series shown in Table I are 3-(1′-phenylpropyl)-4-hydroxycoumarin (Marcoumar) (Jurgens, 1953); 3-(α-naphthyl)-4-hydroxycoumarin (Moraux, Meunier & Mentzer, 1953) and 3-(α-acetonyl-*p*-nitrobenzyl)-4-hydroxycoumarin (G. 23350) (United States, 1953).

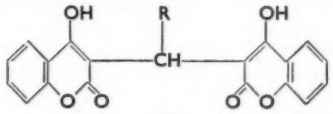
ii. Cyclic acetals (II) in which R_1 is an aryl group, and R_2 and R_3 are alkyl groups.



II

The most important compound of this type is 3:4-(2′-methyl-2′-methoxy-4′-phenyl)-dihydropyranocoumarin (cyclocoumarol) (Link, 1944) (Table I).

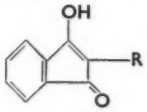
iii. Compounds containing two coumarin ring systems and having the general formula (III).



III

In Table I, examples of this group are methylene-bis-3-(4-hydroxycoumarin) (dicoumarol) (Butt, Allen & Bollman, 1941) and bis-3-(4-hydroxycoumarinyl)-ethyl acetate (ethyl biscoumacetate; Tromexan) (Rosicky, 1944).

iv. Derivatives of 1:3-indanedione (IV). These compounds, although not coumarin derivatives, are so closely related to the latter both in chemical structure and mode of action (Walker & Hunter, 1954) that they are usually classified along with coumarin anticoagulants.



IV

Of these, 2-phenylindane-1 : 3-dione (see Table I) has achieved considerable clinical importance, and 2-(diphenylacetyl)-indane-1 : 3-dione (Dipaxin) is receiving extensive clinical trial at present.

2. Relationship between Chemical Structure and Anticoagulant Activity

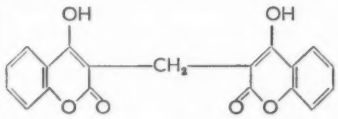
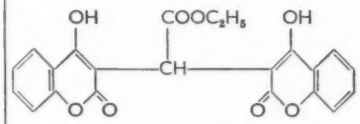
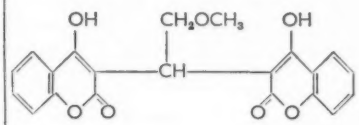
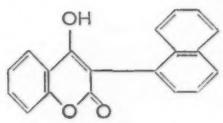
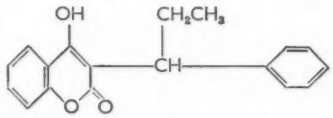
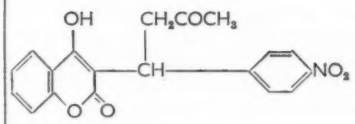
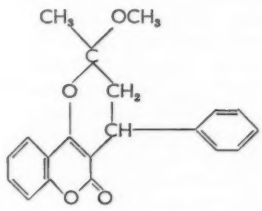
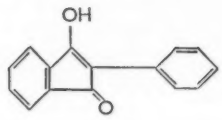
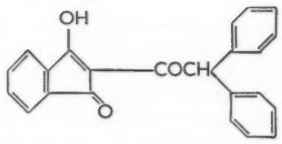
While several interesting relationships between chemical structure, physical properties and anticoagulant activity have been pointed out, we are still unable to define the minimum structural characteristics that are required to confer anticoagulant powers on a molecule (Shepherd, Walker & Hunter, 1954).

Following the identification of dicoumarol as the agent responsible for the haemorrhagic sweet clover disease of cattle, numerous attempts were made to modify the structure of the dicoumarol molecule to produce anticoagulants of enhanced therapeutic value. The fact that ethyl biscoumacetate (with its low water solubility) is a potent anticoagulant, while its water-soluble parent (carboxylic acid) is inactive, suggested to Veldstra, Wiardi & Alberda (1953) that anticoagulant activity might be related, to some extent at least, to the lipophilic properties of the molecule. In support of this claim they showed that simple aliphatic ethers of 2 : 2-bis-(4-hydroxycoumarinyl-3)-ethanol (Table I) were comparable in anticoagulant activity to dicoumarol.

On the basis of spectrographic and potentiometric measurements, Knobloch, Kakac & Macha (1952) have demonstrated a tautomeric equilibrium between 4-hydroxycoumarin (V) and 2-hydroxychromone (VI). They claim that the latter structure

V VI

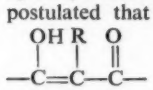
TABLE I. COUMARIN ANTICOAGULANT DRUGS OF CLINICAL IMPORTANCE

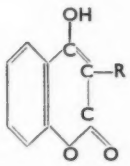
Compound	Structural formula
Dicoumarol (Methylene-bis-3-(4-hydroxycoumarin))	
Ethyl biscoumacetate (Bis-3-(4-hydroxy-coumarinyl)-ethyl acetate)	
Methyl ether of 2:2-bis-(4-hydroxy-coumarinyl-3)-ethanol	
3-(α-Naphthyl)-4-hydroxycoumarin	
Marcoumar (3-(1'-Phenylpropyl)-4-hydroxycoumarin)	
G.23350 (3-(α-Acetonyl-p-nitrobenzyl)-4-hydroxycoumarin)	
Cyclocoumarol Cumopyran (3:4-(2'-Methyl-2'-methoxy-4'-phenyl)-dihydropyrano-coumarin)	
Dindevan (2-Phenylindane-1:3-dione) (as enol)	
Dipaxin (2-(Diphenylacetyl)-indane-1:3-dione) (as enol)	

predominates in the case of bis-(4-hydroxycoumarin) derivatives and attribute the anticoagulant effect of these to fully dissociated anions of the chromone type at pH 7–8.

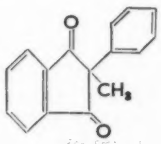
The work of Stahmann, Wolff & Link (1943) demonstrated that anticoagulant activity was not confined to the bis-(4-hydroxycoumarin) type of compound, but could arise in 4-hydroxycoumarin itself when suitable groups were substituted on carbon atom 3. It was found that the anticoagulant effect increased as the chain-length of the alkyl substituent increased, and that aryl groups imparted greater activity than alkyl groups.

When the ring oxygen atom of 4-hydroxycoumarin is omitted, indane-1 : 3-dione is obtained, and it has been shown that several derivatives of this substance are potent anticoagulants (Kabat, Stohlmann & Smith, 1944; Jurgens, 1953).

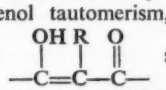
In seeking a correlation between chemical structure and pharmacological activity among anticoagulants of the hydroxycoumarin and indanedione types, Mentzer (1948) postulated that the anticoagulant activity arose from the  system present in these compounds, for example,

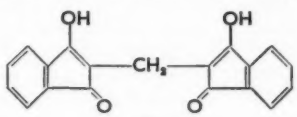


The case of 2-phenyl-2-methylindane-1 : 3-dione (VII), which is completely devoid of any anticoagulant action, seems to

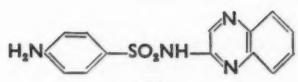


VII

support this theory, since the presence of the methyl group prevents the occurrence of keto-enol tautomerism, thus precluding the development of the  system (Meunier, Mentzer & Molho, 1947). In conflict with Mentzer's theory, however, are 2 : 2'-methylene-bis-indane-1 : 3-dione (VIII) which, while containing the above atomic grouping, has no anticoagulant activity, and 2-sulphanilamidoquinoxaline (IX) which is a potent anticoagulant although it does not possess the required grouping.



VIII

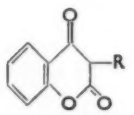


IX

These observations on the validity of Mentzer's theory also apply to that of Knobloch, Kakac and Macha.

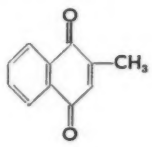
3. Vitamin K and Anticoagulant Action

A subject which has received considerable attention is the relationship of coumarin drugs to the natural and synthetic forms of vitamin K. It is well known that vitamin K deficiency in mammals gives rise to hypoprothrombinaemia, and the resulting haemorrhagic condition bears a very close resemblance to that which is produced by the administration of coumarin anticoagulants. Furthermore, the haemorrhage caused by coumarin drugs is rapidly cured by treatment of the animal with vitamin K_1, 2-methyl-3-phytyl-1 : 4-naphthaquinone, suggesting that there exists between the drugs and the vitamin an antagonism resembling that observed by Woods & Fildes (1940)[1] between sulphonamides and *p*-aminobenzoic acid and having a similar origin, namely, a structural similarity between the drug and the essential body metabolite, vitamin K. The structural relationship is most readily appreciated by comparison of formula (X) representing the keto form of 3-alkyl-4-hydroxycoumarin and that of the synthetic vitamin, 2-methyl-1 : 4-naphthaquinone (XI) (menadione).



keto form

X



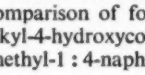
XI

Since vitamin K is required, possibly as a coenzyme, for the synthesis of prothrombin by the liver, it is now believed that coumarin drugs may act as competitive inhibitors which prevent the utilization of the vitamin by one of the enzymes involved in this synthesis. The formation of prothrombin and probably also of Factor VII (Koller, Loeliger & Duckert, 1951) and of Factor X (Koller, communication to Fourth Congress of the European Society of Haematology, Amsterdam, 8–12 September, 1953; Hunter & Walker, 1954) is thus diminished, resulting in interference with the blood coagulation process.

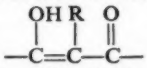
This theory of anticoagulant action, though attractive, has not yet been completely accepted, principally because it requires the antagonism between vitamin K and coumarin drugs to be competitive—that is, the amount of vitamin necessary to neutralize the effect of the drug should be proportional to the amount of drug administered over a wide range of dosage. In practice a stage is soon reached at which even massive doses of the vitamin cannot counteract the effect of the drug. Nevertheless this theory is generally accepted as a working hypothesis to explain the action of anticoagulants, a fact which is emphasized by the increasing frequency with which these drugs are described as antivitamins K. Such a term is misleading, however, since compounds exist which are antivitamins K with respect to certain micro-organisms for which vitamin K is a growth factor, but which are not anticoagulants, e.g., 2 : 3-dichloro-1 : 4-naphthaquinone (Table II).

The belief that coumarin anticoagulants produce their effect by competing with vitamin K is supported by the fact that numerous naphthaquinone derivatives, which are even more closely related structurally to vitamin K than are coumarin compounds, possess anticoagulant activity. A study of such naphthaquinone derivatives, however, is of only limited value so far as the elucidation of relationships between anticoagulant effect and chemical structure is concerned, since, as may be seen from Table II, three types of physiological response may be evoked by very slight changes in chemical structure : (i) anticoagulant action, (ii) vitamin K activity, and (iii) antivitamin K activity. The complexity of these relationships may be appreciated from the examples quoted in Table II. If we take 1 : 4-naphthaquinone as starting point, it appears that the introduction of small substituents (CH_3 or OH) favours vitamin K activity, while large substituents (larger than 5 carbon atoms) give rise to anticoagulant activity. Exceptions are 2-*n*-butylaminomethyl-3-hydroxy-1 : 4-naphthaquinone which has a long chain but neither type of activity, and 2-methoxy-1 : 4-naphthaquinone which, having a short-chain substituent, acts as an anticoagulant in mammals and as antivitamin K in certain fungi.

Five of the compounds listed in Table II contain the



configuration, and are marked with an asterisk; two are anticoagulants, two have vitamin K activity, and one has neither type of activity. Thus, as pointed out by Smith (1950), it appears that, in the naphthaquinone series, the nature of the substituent R is as important as the presence of the



system in conferring anticoagulant activity. While the structural similarity and physiological antagonism between anticoagulants of the coumarin and naphthaquinone type on the one hand and vitamin K-active naphthaquinones on the other suggest very strongly that anticoagulants act by interfering with utilization of the vitamin by the animal, the fact that no simple competitive antagonism can be demonstrated suggests that some other factor or factors are involved, and that the methods of study used at present are inadequate for the purpose. For example, the one-stage (Quick) " prothrombin " time test which has been used widely to determine the action of anticoagulant drugs is a crude measure of the complicated changes which result in the conversion of prothrombin to thrombin.

It is now accepted that the principal results of the administration of anticoagulant drugs in therapeutic doses are depression of Factors VII and X and, to a lesser degree, of prothrombin. By improved methods such as the thromboplastin-generation test (Biggs & Douglas, 1953a) and estimates of Factor VII and Factor X, it may be possible to demonstrate a true antagonism between the drugs and the vitamin. It seems unlikely that a precise correlation between chemical structure and anticoagulant action will be achieved until these additional factors have been elucidated.

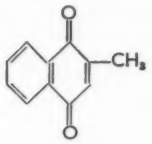
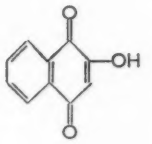
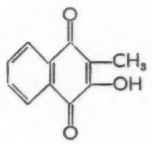
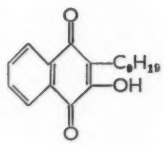
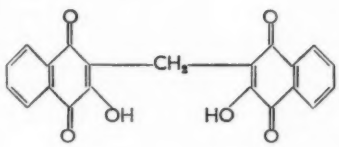
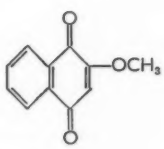
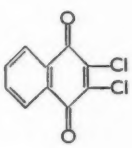
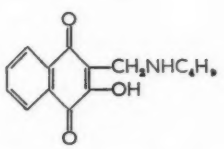
4. Distribution and Metabolism of Coumarin Derivatives in the Body

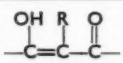
The distribution and metabolism of coumarin anticoagulants in the body have been investigated by colorimetric, spectrophotometric and radiochemical procedures.

A colorimetric method for the determination of ethyl biscoumacetate or dicoumarol in blood plasma and serum, introduced by Pulver & von Kaulla (1948), depends on the ability of these compounds to unite with diazotized *p*-nitrani-

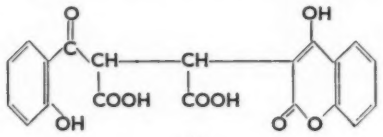
[1] See also Woods, *Brit. med. Bull.* 1953, 9, 122.—Ed.

TABLE II. STRUCTURE-ACTIVITY RELATIONSHIPS OF SOME VITAMIN K ANALOGUES

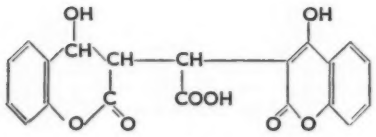
Compound	Structure	Type of activity	Reference
2-Methyl-1:4-naphthaquinone (menadione)		Vitamin K	Seegers (1951)
2-Hydroxy-1:4-naphthaquinone*		Vitamin K	Smith, Fradkin & Lackey (1946) Burger (1951)
2-Methyl-3-hydroxy-1:4-naphthaquinone* (phthiocol)		Vitamin K	Meunier (1949)
2-(2-Methyloctyl)-3-hydroxy-1:4-naphthaquinone*		Anticoagulant	Smith *et al.* (1946) Smith (1947)
Methylene-bis-2-(3-hydroxy-1:4-naphthaquinone)* (diphthiocol)		Anticoagulant	Seegers (1951) Meunier (1949)
2-Methoxy-1:4-naphthaquinone		Anticoagulant Antivitamin K	Meunier (1949)
2:3-Dichloro-1:4-naphthaquinone		Antivitamin K	Woolley (1945)
2-*n*-Butylaminomethyl-3-hydroxy-1:4-naphthaquinone*		Inactive	Smith (1950)

* This compound contains  configuration; see also p. 58 of text.

line at pH 6 to form a yellow dye. The method is unsuitable for urine or body tissues and has the disadvantage that metabolic products of the drugs can also react with the diazo reagent, thus introducing an appreciable error. Two such substances, both non-hypoprothrombinaemic, designated Tromexan acid A and Tromexan acid B (to which Pulver and von Kaulla have provisionally assigned structures—(XII) and (XIII), respectively), were isolated from the urine of rabbits, following oral administration of ethyl biscoumacetate.



XII



XIII

For the determination of dicoumarol Axelrod, Cooper & Brodie (1949) developed a spectrophotometric method which is specific in that metabolites of the drug are not simultaneously determined. The method involved extraction of the dicoumarol from plasma or urine with heptane, re-extraction into dilute alkali, measurement of the extinction at 315 mμ. and determination of the concentration from a previously prepared standard curve. A similar principle was applied to the determination of phenylindanedione by Lowenthal & Ogilvie (1952) and by Schulert & Weiner (1954).

Lee, Trevoy, Spinks & Jaques (1950) have studied the metabolism and excretion of radioactive dicoumarol, while Hausner, Shafer, Corson, Johnson, Trujillo & Langham (1951) have used radioactive dicoumarol and ethyl biscoumacetate, the labelling in each case being with ^{14}C in the methylene bridge. The latter workers carried out paper partition chromatographic examinations of urine and plasma from rats receiving these labelled drugs and were able, using an autoradiographic method, to detect seven metabolites of dicoumarol and at least three of Tromexan.

The results of studies by these various chemical techniques are in good agreement. Thus it is clear that dicoumarol is more slowly absorbed and less rapidly excreted than ethyl biscoumacetate; in these respects phenylindanedione occupies an intermediate position (Schulert & Weiner, 1954). The slow excretion of dicoumarol is probably due to its extensive localization on plasma proteins, particularly albumin (Weiner, Shapiro, Axelrod, Cooper & Brodie, 1950), so that only a low concentration of drug is available to drive the biochemical reactions which convert it to inactive metabolic products. The extent to which ethyl biscoumacetate and phenylindanedione are localized on plasma proteins does not appear to have received detailed study. Although the time of maximum absorption and maximum tissue concentration does not quite correspond with the time of maximum prolongation of the one-stage Quick test, there is a rough correlation between the duration of this action and the persistence of dicoumarol in plasma (Weiner, 1955). It may well prove to be the case that there is a better correlation between blood coumarin levels and the concentrations of Factor X or of prothrombin when estimated by the two-stage area method of Biggs & Douglas (1953b). It is interesting that 2-methyl-1 : 4-naphthaquinone does not affect the rate of excretion of dicoumarol; this suggests that the vitamin does not exert its antihaemorrhagic effect by altering the metabolic pathways of the drug in the body (Weiner *et al.* 1950). However, Lee *et al.* (1950) have shown that the presence of vitamin K apparently interferes with the fixation of dicoumarol by the liver—the drug, which normally accumulates in considerable amounts in that organ, being apparently displaced from it more rapidly; they suggest, therefore, that the time during which dicoumarol remains in the liver is related to its effectiveness in interfering with prothrombin formation.

The time-lag between the administration of coumarin anticoagulants and prolongation of the one-stage Quick test has led to the belief that the drug may undergo chemical transformation in the body to produce a degradation product which is the true haemorrhagic agent. Although the coumarin ring system is apparently rather stable in vivo (Williams, 1947), it is known that dicoumarol can be converted under laboratory conditions to salicylic acid, the very slight anticoagulant effect of which is antagonized by vitamin K (Link, Overman, Sullivan, Huebner & Scheel, 1943). However, salicylic acid, which, if formed, should be excreted in the urine, has not been detected following the administration of dicoumarol (Lester, 1944); moreover, even if a therapeutic dose of dicoumarol were quantitatively converted to salicylic acid, the amount of the latter would be insufficient to account for the observed anticoagulant effect. It also seems unlikely that salicylic acid or any of its derivatives would be formed from phenylindanedione by degradation of the latter in the body.

The probable explanation for the delayed action is that a certain amount of time is required for the consumption of prothrombin and the other factors already present.

5. Discussion

In spite of the very large number of coumarin drugs already studied, no satisfactory correlation between chemical structure and anticoagulant action has yet been demonstrated. This is perhaps not surprising in view of the influence of small structural changes on such variables as solubility, rate of absorption, ease of distribution, degree of binding by tissues or plasma proteins, and rate of detoxication and excretion. The antimetabolite theory of anticoagulant action, which has gained wide acceptance, is based on the structural similarity and observed biological antagonism between coumarin compounds and vitamin K, although this antagonism is not competitive over a wide range of dosage as is that between the sulphonamides and *p*-aminobenzoic acid. It is possible, however, that a completely competitive antagonism might yet be demonstrated if the essential step of the series of reactions constituting the coagulation process could be studied as an isolated system in vitro. This and the fact that coumarin anticoagulant drugs apparently exert important effects on Factor VII and Factor X as well as on prothrombin synthesis suggest that further insight into the coagulation mechanism and into anticoagulant therapy may be derived from a study of the interactions of coumarin drugs, vitamin K, and specific plasma proteins.

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CHEMISTRY AND MODE OF ACTION OF HEPARIN AND RELATED COMPOUNDS

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1 Heparin
- *a* Chemistry
- *b* Variation of characteristics
- *c* Molecular size
- *d* Other physical characteristics
- *e* Relation between chemical structure and anticoagulant activity
- *f* Reaction with bases
- *g* Metachromatic reaction with basic dyes
- *h* Assay
- *j* Biological behaviour
- *k* Mode of anticoagulant action

2 Compounds related to heparin
- *a* Mode of anticoagulant activity
- *b* Anticoagulant potency
- *c* Duration of anticoagulant activity

3 Heparin and heparinoids
- *a* Metabolism and excretion
- *b* Toxicity in animal experiments
- *c* Clinical side-effects

References

The presence of material in mammalian tissues capable of interfering with blood coagulation appears to have been known since before the turn of the present century. It was then commonly referred to as "antithrombin". Bayliss discussed the clinical possibilities of the use of such material in 1915. However, it was not until 1916 that McLean, while attempting to purify a liver phosphatide known to possess thromboplastic properties, produced an extract showing the apparently paradoxical effect of retarding the clotting of normal blood.

Subsequent progress in the isolation, chemical identification and development of heparin for the therapy of thrombosis has been reviewed previously by Howell (1928), Best (1940), Jorpes (1946) and Seegers (1951). In the present account, further expansion in knowledge concerning the nature and structure of heparin will be discussed. In addition, the effect of heparin and its analogues on biological systems not related directly to the coagulation mechanism will be considered, since such work has thrown oblique light upon the fundamental mechanism of activity of these compounds as a group.

1. Heparin

a. Chemistry

Although it is customary to regard heparin as a well-defined entity, the advance of knowledge concerning the family of naturally occurring mucopolysaccharides, to which heparin belongs, has made its chemical and physical definition difficult. The *British Pharmacopoeia* (General Medical Council, 1953) describes heparin as "The sodium salt of a complex organic acid present in mammalian tissues, and having the characteristic property of delaying the clotting of shed blood". Most modern extraction procedures for the isolation of material corresponding with this definition are modifications of the original procedure developed by Charles & Scott (1933). Animal tissues (usually beef lung) are extracted with alkaline ammonium sulphate or potassium thiocyanate, the extract precipitated with acid, fat removed by treatment with solvents, and protein removed by protein precipitants or tryptic digestion. This gives a crude material containing 5–15% of biologically active material. Subsequent purification is effected by treatment with alcohol, acetone or brucine. Finally, heparin is obtained as its calcium, barium or sodium salt. The last of these is the most commonly prepared commercially.

Purification via the barium salt (Charles & Scott, 1936) advanced the investigation of the chemical structure of heparin, but the conclusive demonstration that it was mucopolysaccharide in nature was made by Jorpes (1935), who confirmed Howell's earlier contention that the material contained uronic acid and also demonstrated the presence of hexosamine. The uronic acid was at first thought to be galacturonic acid, suggesting a close relationship to chondroitin sulphate, but Wolfrom & Rice (1946) later showed it to be glucuronic acid.

The major Scandinavian contribution, however, was the finding that the ester sulphate content of heparin varied between about 7% and 14% (for references, see Jorpes, 1946), thus exceeding that of any other known constituent of the tissues.

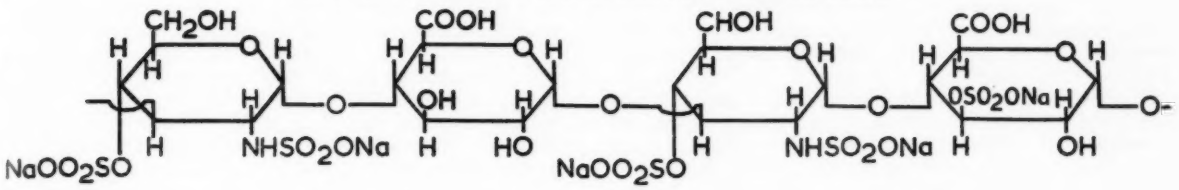
At this stage, the repeating unit in the molecule was thought to be a disaccharide consisting of glucosamine and glucuronic acid. Elementary analysis of the barium salt of heparin gave values of C, 20.12; N, 2.03; S, 11.44; H, 2.61; hexuronic acid, 17.8; hexosamine, 17.8; and barium, 23.25%, with a molar ratio of glucosamine to glucuronic acid to ester sulphate to barium of 2:2:5:2.5 (Wolfrom, Weisblat, Karabinos, McNeely & McLean, 1943). Little was known concerning the exact mode of linkage of uronic acid to amino sugar, nor was the disposition known of the ester sulphate groups.

It had long been held that the amino sugars of all naturally occurring mucopolysaccharides were *N*-acetylated (Fürth, Herrmann & Scholl, 1934), and it was assumed that this was true of heparin also. It followed, from the nature of the constituent uronic acid and amino sugar in the postulated disaccharide unit, that heparin was probably a polysulphuric acid ester of mucoitin.

Wolfrom and his co-workers (Wolfrom *et al.* 1943; Wolfrom & McNeely, 1945) noted that exposure to very mild acidity caused heparin to lose its anticoagulant activity at a rate proportional to the rate of appearance of a free amino group, and they suggested that the nitrogen linkage was the seat of its biological activity. Recently, Jorpes, Boström & Mutt (1950) and Meyer & Schwartz (1950) have produced evidence that the amino group of the glucosamine moiety is sulphated and not acetylated, thus distinguishing heparin from mucoitin sulphate and demonstrating that heparin is unique among the family of mucopolysaccharides in this respect.

Meanwhile, periodate oxidation studies on sodium heparinate and its degradation products provided provisional

FIG. 1. DETAILED STRUCTURE OF SODIUM HEPARINATE



evidence of its detailed structure (Wolfrom, Montgomery, Karabinos & Rathgeb, 1950). This was confirmed by further work on the periodate oxidation of heparin, following the use of 2:4-dinitrofluorobenzene as an amino-blocking reagent and the use of the toluene-*p*-sulphonate ("tosylate") technique of Oldham & Rutherford (1932) for terminal hydroxyl groups (Neely, 1953). The final structure proposed is shown in fig. 1.

Although a definite structure can thus be assigned to a highly purified preparation from a definite source, there are various indications that, viewed from a wider standpoint, it may not be possible to regard heparin as a single well-defined chemical compound.

b. Variation of Characteristics

It was shown by Jaques & Waters (1940) and by Jaques, Waters & Charles (1942) that heparin prepared from the livers of different animal species (dog, ox, pig and sheep) varied in activity, although elementary analyses showed closely similar chemical composition. Although Wolfrom, Karabinos, Smith, Ohlinger, Lee & Keller (1945) found these results unacceptable, similar findings have been reported by Marbet & Winterstein (1952). Recently, examination by paper chromatography of heparins isolated from these different animal sources has been reported to show variations between species of the components separable by this method (Bell, 1953).

Even with material derived from a single species and organ (beef lung) it has been found that different commercial samples consist of varying mixtures of mono-, di- and trisulphuric acid esters (Jorpes & Gardell, 1948).

c. Molecular Size

Even purified samples of beef heparin examined for homogeneity of molecular size have been found to be polydisperse by a variety of techniques. Thus two, three or more fractions of varying molecular size and activity have been found to be separable by ultracentrifugation (Grönwall, Ingelman & Mosimann, 1945); by electrophoresis (Wolfrom & Rice, 1947; Jensen, Snellman & Sylvén, 1948) and by Craig's counter-current distribution technique (O'Keeffe, Russo-Alesi, Dolliver & Stiller, 1949). Most estimates assign a molecular weight of 16,000–17,000 as an average for the distribution of molecules in the preparations examined. By combining ultracentrifugation with light-scattering measurements, Neely identified two fractions, one with relatively low anticoagulant activity and a molecular weight of 16,000, the other with high activity and a molecular weight of 50,000. If one accepts a molecular weight of 16,000 as characteristic of the material usually isolated, and the repeating unit shown in the figure above, these would indicate heparin to be a polymer containing about 52 sugar units. There is little information available concerning the disposition of these units (i.e. whether they are arranged as branched or straight chains), although the studies of Grönwall *et al.* (1945) on the ultracentrifugation and diffusion of heparin suggest that the molecule is highly asymmetrical.

It is possible that the apparent heterogeneity of heparin may result from the manipulations involved in its isolation. It has recently been shown that cleavage of polysaccharide chains occurs in an alkaline medium in the presence of oxygen (Bottle, Gilbert, Greenwood & Saad, 1953), and it is known that *O*-sulphate groups are relatively labile towards alkali (Meyer & Schwartz, 1950), so it is possible that the end-product of the usual isolation and purification procedures is a considerably modified version or versions of the molecule as it exists in the tissues.

d. Other Physical Characteristics

Beef heparin, as ordinarily isolated, is strongly dextrorotatory, suggesting α-glycoside linkages between the component saccharide units. The sodium salt of beef heparin of the highest degree of purity has been reported to have a specific optical rotation of $+65°$, a sulphur content of 13.8%, and an anticoagulant activity of 130 i.u./mg. (Jorpes, 1946). Commercial samples with lower sulphur content and less activity have been found to show lower optical activity (Walton & Ricketts, 1954). There appears to be little justification for the terminology suggested by Marbet & Winterstein (1952) of "α-heparins" for products which are dextrorotatory and show high anticoagulant activity, and "β-heparins" for products from the same sources which are laevorotatory and show lower anticoagulant activity, since these authors appear to be unable to distinguish the latter products from chondroitin sulphate except in respect of anticoagulant activity (also see the comment below).

The presence of ester sulphate groups confers a strong electro-negative charge on heparin. This is shown by the high electrical conductance and the high cataphoretic mobility of heparin in an electrical field (Wilander, 1938). Although these features suggest marked dissociation of the reactive groups, the osmotic pressure of heparin solutions is extremely low and, in contrast with citrate or oxalate solutions, heparin has no osmotic influence on the red cell.

e. Relation between Chemical Structure and Anticoagulant Activity

The international standard for heparin contains, by definition, an activity of 130 i.u./mg. Most commercial samples of British, Swedish and American manufacture contain 90–120 i.u./mg. Efforts have been made to correlate chemical structure with anticoagulant activity. The present evidence (discussed more fully in section 1k) indicates that heparin intervenes in the complex series of protein–protein interactions which comprises the blood coagulation mechanism by interaction

with one or more of the component "clotting proteins". This reactivity with proteins has been ascribed to the electronegative charge conferred by the acidic radicals of the heparin molecule (Demole & Reinert, 1930; Bergström, 1935; Jorpes, 1946).

The available acidic radicals on the heparin molecule are: the free carboxyl groups of the uronic acid residues, the *O*-sulphate ester groups distributed on both uronic acid and amino-sugar components, and the amino-sulphate (*N*-sulphate) groups of the amino sugars. The almost complete dissociation of sulphate groups at physiological pH, compared with the relatively slight dissociation of carboxyl groups, makes it likely that the former are the chief contributors to the strong charge borne by the whole molecule.

The importance of the sulphate group in determining anticoagulant activity was shown when it was found that, for heparin from any one biological source, anticoagulant activity rose in rough correspondence with increase of sulphur content (Jorpes & Bergström, 1939). It had also been shown earlier by Bergström (1936) that the sulphation of other polysaccharides, such as starch, cellulose, chitin, etc., conferred some heparin-like anticoagulant activity. This was confirmed by Chargaff, Bancroft & Stanley-Brown (1936), who demonstrated further that the sulphation of even unrelated macromolecular polymers, such as polyvinyl alcohol, produced similar activity.

The free carboxyl group of the glucuronic acid moiety evidently makes little contribution to anticoagulant activity, since the sulphation of carboxylated polysaccharides does not confer any further activity (Karrer, Usteri & Camerino, 1944; Ricketts & Walton, unpublished observations).

Evidently *O*-sulphate groups alone are not responsible for the uniquely high activity of heparin, since the maximum activity attained in synthetic polysaccharide sulphates possessing these groups is usually less than 30 i.u. of heparin/mg. In consequence, the *N*-sulphate group distinguishing heparin from its analogues has received considerable attention. Efforts have been made to synthesize this group by the sulphation of chitosane, a naturally occurring polymer of glucosamine (Ricketts, 1953; Doczi, Fischman & King, 1953), or by the *N*-deacetylation of chitin and chondroitin sulphate followed by sulphation (Wolfrom, Shen & Summers, 1953). Ricketts reported no increase in activity, but the other investigators reported activities of 48–57 i.u. of heparin/mg., and Doczi and his colleagues found this increase to be associated with a preponderance of *N*-sulphate over *O*-sulphate groups.

However, Foster, Martlew & Stacey (1953) found that some heparin preparations with a high proportion of *N*-sulphate groups possessed low activity. Moreover, Jorpes, Boström and Mutt showed that alkaline degradation of heparin with ammonium hydroxide did not affect the amino linkage to any great extent but did hydrolyse some of the *O*-sulphate ester groups. The alkali-degraded heparin was biologically inactive. Finally, it has been claimed by Marbet and Winterstein that a special variety of chondroitin sulphate (their so-called "β-heparin") isolated from beef and sheep lung showed an anticoagulant activity of 36–70 i.u. of heparin/mg., although it was found to possess an acetylated amino group on its amino sugar (galactosamine). In addition, on further sulphation without prior *N*-deacetylation, it was reported to attain an activity of 160 i.u. of heparin/mg. (i.e. an even higher activity than their purest "α-heparin" from the same biological source).

It appears possible, therefore, that undue attention has been paid to the sulphamino group as such and that the greater activity of heparin as compared with its analogues may be determined, not by the presence of *N*-sulphate groups, nor by their proportion to *O*-sulphate groups, but by a particular molecular configuration brought about by internal linkages through these groups (Foster, Martlew & Stacey, 1953). Differences in the fine molecular structure, producing variations in molecular size and shape, rather than the proportions of specific groupings on the molecule, may also account for the fact that, when heparins from different species are compared, the correspondence between activity and sulphur content disappears. The relation between molecular size and configuration and biological activity will be considered again in discussing heparin analogues.

f. Reaction with Bases

Heparin forms compounds with organic bases (benzidine, octylamine); with alkaloids (quinine, brucine); with materials in which basic amino acids predominate (protamines, histones and basic polyamino acids such as polylysine), and with a variety of basic dyes. Interest has recently arisen in the interaction of heparin with complex bases of pharmacological importance such as aureomycin (Waisbren & Glick, 1950), histamine, antihistamines and histamine releasers (Riley, 1953; Mota, Beraldo & Junqueira, 1953). Compounds of heparin with the basic local anaesthetics, butacaine and procaine, have been investigated by Loomis, Jesseph & Beyer (1952) and by Loomis & Jesseph (1952). It is claimed that the butacaine–heparin compound dissociates slowly in the tissues, when injected subcutaneously or intramuscularly, giving prolonged anticoagulant effect without painful haematomata or toxic effects.

When the free basic groups of the various compounds listed above react with the acidic groups of heparin, the latter's anticoagulant activity is nullified. This is the basis of the use, in clinical practice, of protamine or of the basic dye, toluidine blue, as antidotes to heparin. It is insufficiently appreciated, however, that some of these basic substances themselves show anticoagulant activity (for example, see Rubini, Becker & Stahmann, 1953) and that, if present in excess of the amount required to neutralize heparin, they may produce an apparently paradoxical increase in the coagulation time (Jaques, Charles & Best, 1938).

g. Metachromatic Reaction with Basic Dyes

On the addition of increasing concentrations of heparin to a constant dilute concentration of toluidine blue, the colour of the dye changes from its normal (orthochromatic) blue through purple to reddish violet (metachromatic) shades. Metachromasia on the addition of heparin also occurs with other basic dyes, especially with members of the oxazine, phenazine and thiazine series.

The reaction is not specific for heparin, being given with comparable intensity by other sulphated polysaccharides (both naturally occurring and synthetic) and with less intensity by polycarboxylates, polyphosphates and silicates. Quantitative investigation of the basis of the reaction has shown that the intensity of metachromasia is directly related to the number and the dissociation characteristics of the attached acidic radicals but not to the nature, disposition or number of the constituent units for glucose polymers containing more than four saccharide units; that is to say, the

intensity of metachromasia, for all practical purposes, can be held to be directly related to sulphur content but unrelated to the degree of polymerization of acidic polysaccharides (Walton & Ricketts, 1954).

Although the metachromatic reaction can be made sufficiently sensitive to detect concentrations of sulphated polysaccharides as low as 2–5 μg./ml. in pure solution, the presence of proteins or of other materials capable also of binding the dye seriously impairs this sensitivity. Nevertheless the reaction has been used for the detection of material alleged to be heparin in the tissues (Holmgren & Wilander, 1937; Jorpes, Holmgren & Wilander, 1937; Asplund, Borell & Holmgren, 1939); in tissue extracts (Jaques, Bruce-Mitford & Ricker, 1947), and quantitatively in tissue fluids (Copley & Schnedorf, 1941; Jaques, Monkhouse & Stewart, 1949; Monkhouse & Jaques, 1950). The fallacy of uncritically accepting metachromasia in certain situations as a specific reaction for heparin has been pointed out by Jaques (1952) and by Walton (1954) in different contexts.

h. Assay

Assay methods for heparin have been reviewed by Jaques & Charles (1941), Jalling, Jorpes & Lindén (1946), Jorpes (1946) and Blömback, Blömback, Corneliusson & Jorpes (1953). The multiplicity of methods available suggests in itself that no one of them is completely satisfactory. Jaques, Waters & Charles (1942) found that heparins obtained from different species showed different anticoagulant activities in comparison with one another when tested by several methods.

It is generally agreed that the anticoagulant potency of heparin intended for clinical use should be measured in biological systems incorporating the clotting mechanism of normal blood. Although many methods use plasma in one form or another, the authors with widest experience of heparin assay advocate the preferential use of whole blood. The *British Pharmacopoeia* (General Medical Council, 1953) recommends a method devised by Adams (1950) that employs sulphated whole beef blood.

Two methods based upon physico-chemical reactions independent of blood coagulation have been proposed. These are titration with protamine (Jaques & Waters, 1941) and the colorimetric reaction with toluidine blue (MacIntosh, 1941). The intensity of interaction of both these bases with polysaccharide sulphates is directly related to the sulphur content of the latter (Walton & Ricketts, 1954; Saddington & Walton, unpublished results). But sulphur content correlates only roughly with anticoagulant activity, both for heparin as discussed above and for other polysaccharide sulphates (Ricketts, 1952a). Neither of these methods can be accepted, therefore, as reliable assay techniques.

j. Biological Behaviour

Heparin shows a remarkable diversity of biological activities. Among these may be mentioned:

i. Effect upon enzymes. Heparin has been shown to inhibit the activity of fumarase (Fischer & Herrmann, 1937); trypsin (Horwitt, 1940); hyaluronidase (McClean, 1942; Astrup & Alkjaersig, 1950); streptococcal ribonuclease (Sherry, Johnson & Tillett, 1949); cathepsin (Klingenberg, 1951); ribonuclease and deoxyribonuclease from mammalian tissues (Roth, 1952) and lysozyme (Kerby & Eadie, 1953). Halse (1949a) and Halse, Philipp & Ruf (1950) have claimed to have shown that while heparin inhibits the activity of plasmin in vitro it accelerates the rate of lysis of thrombi in vivo.

ii. Effect upon cells and tissues. Heparin has long been known to inhibit mitosis (for references, see Paff, Sugiura, Bocher & Roth, 1952). The in-vitro growth and differentiation of marine eggs (Heilbrun & Wilson, 1949), bacteria (Warren & Graham, 1950) and mammalian cells in tissue culture (Balazs & Holmgren, 1949) are arrested by low concentrations of heparin but there is no evidence that the latter interferes with normal growth in the intact animal.

iii. Effect upon proteins. Heparin, like other polyanionic colloids, can be made to form insoluble salts with proteins at reactions below their isoelectric points (Fischer, 1931; Gorter & Nanninga, 1953; Clarke & Monkhouse, 1953). But the reactions with proteins of greater biological interest are those occurring at physiological pH, which are not necessarily accompanied by visible precipitate formation. Changes in the electrophoretic pattern of the plasma proteins on the addition of heparin were studied by Chargaff, Ziff & Moore (1941). These authors used heparin at unphysiologically high concentration (4%), which makes their conclusions of limited interest. Hoch & Chanutin (1952), using much lower concentrations, found that amounts of heparin insufficient to prevent blood clotting did not influence the electrophoretic pattern. At slightly higher concentrations, heparin formed soluble complexes mainly with components of the β-globulins and possibly also with α-globulin and fibrinogen. The mobilities of these complexes increased with increase in concentration of the anticoagulant. Interaction with albumin and γ-globulin was slight by comparison. The action of heparin on complement is a special example of interaction with the physiologically important "trace" fractions of the plasma globulins. Complementary activity is known to be an aggregate effect of several globulin components. The action of heparin has been shown to be exerted directly upon one or more of these components (Ecker & Gross, 1929; Ecker & Pillemer, 1941), and not mediated through an effect upon the red-cell or haemolytic antibody. The intensity of anticomplementary activity of polysaccharide sulphates has been found to be dependent on both molecular size and charge and to be unrelated to anticoagulant activity (Taylor & Walton, unpublished results).

iv. Effect on plasma lipids and lipoproteins. It was first observed by Hahn (1943) that heparin administered to dogs during an alimentary lipaemia caused "clearing" of the turbidity of the plasma. The effect is not a direct one upon the plasma lipids (since these show little or no reduction in total amount and since heparin does not produce a similar effect on addition to lipaemic plasma in vitro) but is mediated through interaction with a globulin component ("the lipaemia clearing factor") produced in response to the injection of heparin. Alterations in the flotation rates of the lipoproteins of serum following heparin administration are detectable by ultracentrifugation and are reported to be associated with reduced lipid deposition in the walls of the arteries in cholesterol- and fat-fed experimental animals (for reviews in this field, see Gould, 1951; Nikkilä, 1953; Constantinides, Szasz & Harder, 1953; Herzstein, Wang & Adlersberg, 1954).

k. Mode of Anticoagulant Action

Heparin, because of the broad spectrum of its reactivity with globulins, appears to intervene at more than one point

in the reaction chain of blood clotting. The available evidence is considered in relation to the main components of the system.

Antithrombin action. Although it was held at first that heparin was the natural antithrombin, Howell (1928) found that it did not prevent the interaction of purified thrombin and fibrinogen in the absence of serum or plasma. Quick (1938) found that an essential co-factor resided in crude albumin fractions of serum, confirmed Mellanby's observation (1934) that neutral salts were necessary for the reaction, and suggested that it was the co-factor which was identical with plasma antithrombin. The heparin co-factor is now held to be different from plasma antithrombin (see Volkert, 1942). Seegers, Warner, Brinkhous & Smith (1942) suggested that heparin acted catalytically in the presence of its co-factor, increasing the speed of inactivation—but not the total amount of thrombin destroyed. The studies of Lyttleton (1950, 1954) were broadly in agreement with this conclusion. However, it has subsequently been modified (Klein & Seegers, 1950; Seegers, Miller, Andrews & Murphy, 1952), but Biggs & Macfarlane (1953) have, on the basis of Lyttleton's results, criticized the evidence for the modifications. Jaques & Mustard (1940) and Ziff & Chargaff (1940) found crystalline albumin to be free from co-factor activity. Further efforts to isolate and identify the co-factor in serum have been unsuccessful, but Snellman, Sylvén & Julén (1951) isolated a heparin–lipoprotein complex from mast cells. On dissociation from the complex, the lipoprotein moiety showed co-factor activity for the antithrombin effect of the heparin.

Action on prothrombin. Howell suggested that heparin had antiprothrombin as well as antithrombin activity. However, Quick (1936a) and Mellanby both showed that the conversion of prothrombin to thrombin still proceeded in the presence of heparin. Brinkhous, Smith, Warner & Seegers (1939) found that, in the presence of a serum co-factor, prothrombin conversion, like the action of thrombin on fibrinogen, was inhibited. They were unable to decide whether this was due to an antiprothrombic or an antithromboplastic effect of heparin. Feissly & Enowicz (1946) have suggested that the co-factor for the effect described by Brinkhous and his colleagues is not identical with the antithrombin co-factor.

Action on thromboplastin. Thromboplastic activity is currently held to be exhibited severally by tissue extracts and by factors in the platelets and plasma. Although the addition of brain thromboplastin to heparinized plasma reduces its coagulation time, Quick (1936b, 1944) has opposed the view that there is direct interaction between heparin and thromboplastin. But Chargaff, Ziff & Cohen (1940) isolated a thromboplastic lipoprotein from the lung and showed that it was dissociated by heparin which combined with the protein moiety, releasing lipid. There is indirect evidence that plasma thromboplastin may also be lipoproteinous in nature, and Overman (1949) regards the essential action of heparin to be the release, in this fashion, of a lipid fraction which is actively anticoagulant. Interaction between platelets and plasma factors, when plasma is brought in contact with a foreign surface, such as glass, is held to activate plasma thromboplastin.[1] It has been shown that the anticoagulant activity of heparin, in platelet-free plasma obtained in silicone-treated glass-ware, is enhanced, but is subsequently reduced by an amount corresponding with the area of untreated glass with which such plasma is brought in contact (Conley, Hartmann & Lalley, 1950). Stefanini (1953) postulates an antiheparin factor in platelets.

Effects related to the clotting mechanism. Green (1952) has suggested that the antithrombin action of heparin may be mediated through a coupled phosphorylation reaction, or alternatively (Green, 1953) that heparin combines with an oxidized form of some component of the thrombin complex, since reducing agents retard coagulation, whereas, when thrombin itself is reduced, the antithrombin effect of heparin is diminished. Heparin has long been known to inhibit the release of the vasoconstrictor substance ("serotonin") associated with blood clotting (Janeway, Richardson & Park, 1918; Hirose, 1918).

[1] See Ackroyd, p. 21 of this number of the Bulletin.—Ed.

2. Compounds Related to Heparin

Naturally occurring compounds. Sulphated polysaccharides isolated from sources other than mammalian tissues have been found to show heparin-like activity. Thus, compounds derived from algae, such as *Irideae laminarioides* (Chargaff *et al.* 1936) and *Chondrus crispus* (Elsner, Broser & Bürgel, 1937); and from molluscs, such as *Charonia lampas* (Soda & Egami, 1938), *Mactra spissula* and *Artica islandica* (Frommhagen, Fahrenbach, Brockman & Stokstad, 1953), have been studied and, in some cases, claimed to possess greater activity than heparin.

Synthetic compounds. Early efforts to produce synthetic heparin substitutes by the sulphation of starch, cellulose, chitin, etc., were made empirically and were largely unsuccessful, since these compounds produced haemorrhages and other undesirable effects with comparatively low dosages (Piper, 1945). Recently, four heparinoids (as they have come to be known) have been developed and subjected to clinical trial: Paritol (alginic acid sulphate) and Treburon (pectin sulphate) in the USA, Thrombocid (xylan sulphate) in Germany and Dexulate (dextran sulphate) in Great Britain.

In the case of dextran sulphate, efforts have been made to investigate the effect upon its biological behaviour of systematic variation of the molecular characteristics of the compound. These results and those obtained with other heparinoids have thrown interesting side-lights on the activity of heparin.

a. Mode of Anticoagulant Activity

The heparinoids all show heparin-like antithrombin activity. The action of dextran sulphate has been shown to be dependent upon the presence of a co-factor in serum or plasma (Walton, 1952) that is similar to—or identical with—the heparin co-factor (Lyttleton, personal communication).

b. Anticoagulant Potency

None of the heparinoids shows more than one-fifth to one-quarter the activity, by weight, of commercial heparin, in vitro. Potency is influenced by both molecular weight and sulphur content. Dextrans of short chain-length (less than nine glucose units), even when sulphated maximally (18–20% sulphur) attain a heparin activity of only 8–9 i.u./mg. A dextran composed of 17 glucose units and sulphated to the same degree contained 14 i.u./mg. (Ricketts, 1952b, 1954). On the other hand, a range of compounds prepared from a parent dextran containing about 20 glucose units showed little activity until the sulphur content rose above 12%, a sharp increase in activity between 12% and 16%, and thereafter

practically no further increment in activity with increase of sulphation (Ricketts & Walton, 1952). The sulphation of dextrans of progressively larger molecular size also failed to yield greater activity until a very large molecular size was reached (about 6,000 glucose units), when an apparent activity of about 60–70 i.u./mg. was attained (Walton & Ricketts, unpublished observations). The validity of this estimate of activity must be held in question since compounds of this size exert a direct effect upon fibrinogen (see section 3b).

c. Duration of Anticoagulant Activity

Molecular size and the degree of sulphation are again of importance in this connexion. In experimental animals, dextran sulphates composed of less than six glucose units produced only transitory anticoagulant effects, presumably because of rapid escape from the blood stream and urinary excretion (Ricketts, 1952b, 1954). Compounds composed of about 20 glucose units showed slightly greater duration of effect than heparin in equipotent dosage, while, under similar conditions, dextran sulphates of still greater molecular size showed markedly greater duration of effect. Clinical assessment of the anticoagulant effect of the various heparinoids has been reported: for Paritol, by Sorenson, Seifter & Wright (1949), and by Sorenson & Wright (1950); for Treburon, by Hirschboeck & Madison (1950), and by Scholz & Barker (1952); for Thrombocid, by Halse (1949b), and by Schumacher (1950); and for dextran sulphate, by Ricketts, Walton, van Leuven, Birbeck, Brown, Kennedy & Burt (1953). In most cases, the duration of effect was greater than with heparin.

3. Heparin and Heparinoids

a. Metabolism and Excretion

The evidence for the origin of heparin from the tissue mast cells has been reviewed recently by Bürkl (1952) and by Riley (1954). It is doubtful whether heparin ever circulates in normal blood or is released following massive exposure to ionizing radiations, as was at one time suspected (Allen, Sanderson, Milham, Kirschon & Jacobson, 1948), but its presence in the blood of dogs, following anaphylactic shock, has been confirmed (Allen, 1952; Monkhouse, Fidlar & Barlow, 1952). Nothing is known of the fate of intrinsic heparin, but the excretion of injected heparin has been studied by many investigators (see Jaques, Napke & Levy, 1953); 25–50% is recoverable in the urine, depending on the size of the initial dose. Similar urinary excretory patterns are reported for Paritol (Sorenson *et al.* 1949), Treburon (Mangieri, Engelberg & Randall, 1951) and for clinical dextran sulphate (Ricketts, Walton & Saddington, 1954). It is suggested that the heparin unaccounted for by urinary excretion is destroyed by an enzyme, " heparinase ", isolated from the liver by Jaques (1940). Initially this was regarded as a specific enzyme which brought about degradation of the heparin molecule without loss of sulphate groups, but recent studies (Jaques & Keeri-Szanto, 1952) have shown that loss of antithrombin activity is accompanied by loss of metachromatic activity, suggesting that sulphate loss does occur. This loss of metachromasia, the pH optimum of 6.0–6.5, the association of activity only with particulate matter and the fact that the chemically dissimilar compound, Treburon, is also attacked, all suggest non-specific breakdown by the sulphatases known to be present in liver tissue.

The heparinoids also simulate heparin in: (i) producing similar alterations of the electrophoretic patterns of the plasma proteins; (ii) " clearing " lipaemic plasma; (iii) inhibiting the same enzyme systems; (iv) anticomplementary activity; and (v) reacting similarly with the same range of bases and basic dyes. The intensity with which they exhibit these forms of activity (unlike their anticoagulant activity) may exceed that of heparin, weight for weight.

b. Toxicity in Animal Experiments

Most investigators have confirmed the classical studies of Murray, Jaques, Perrett & Best (1936, 1937), who found that even comparatively crude heparin was effective in treating and preventing experimental thrombosis, without serious toxic effects (but see Jewell, Pilkington & Robinson, 1954). Much larger doses were found necessary to prevent platelet agglutination than were needed to inhibit intravascular clotting (Solandt & Best, 1938). Since then it has been reported that some heparin samples actually produce platelet agglutination (Copley & Robb, 1941; Copley, 1948). These observations were confirmed by Fidlar & Jaques (1948), but they found that carefully purified heparin did not behave in this manner, while Quick, Shanberge & Stefanini (1948) found that the effect was less pronounced in humans than in dogs. Vinazzer (1952) reported that certain heparin samples produced a temporary fibrinogenopenia.

Platelet agglutination and fibrinogen precipitation were found to be characteristic properties of the toxic polysaccharide sulphates investigated by Astrup, Piper and their colleagues (for references, see Astrup, 1953). These properties were found, for dextran sulphates, to be related to the molecular size of the compound (Walton, 1951). Dextran sulphates of large molecular size precipitated fibrinogen. When this occurred in plasma in vitro, the suspension stability of the formed elements of the blood was altered, platelets and leucocytes agglutinated and erythrocytes aggregated. Dextran sulphates of small molecular size, on the other hand, formed soluble complexes with plasma proteins (Walton, 1952) and, like purified heparin, produced no effect on the blood cells (Walton, 1953). On intravenous administration of dextran sulphates of large molecular size, fibrinogenopenia and thrombocytopenia ensued and the particles of the dextran sulphate–fibrinogen complex were removed from the circulation by the phagocytic activity of reticulo-endothelial cells in liver, spleen and bone-marrow. It was pointed out that this behaviour is not exclusive to polysaccharide sulphates but has been observed with other miscellaneous macromolecular colloids (Walton, 1954). Similar observations on the storage of xylan sulphates of varying chain-length were made by Husemann, Hoffman, Lotterle & Widersheim (1952).

Dextran sulphates containing about 20 glucose units were free from undesirable effects in vivo, were not stored in the tissues and were therefore judged suitable for clinical use (Ricketts *et al.* 1953).

c. Clinical Side-Effects

Apart from the occasional occurrence of haemorrhage caused by excessive or injudicious therapy, and rare cases of hypersensitivity (see Hauch, 1952), heparin has been regarded as a safe and non-toxic anticoagulant in clinical practice. Recently, a proportion of patients treated with heparin has been reported to show a transient alopecia. Similar effects have

been noted with some of the heparinoids which have also produced diarrhoea (see Hirschboeck, Madison & Pisciotta, 1954). No explanation of these side-effects has been offered. However, Odeblad, Boström and their colleagues (Odeblad & Boström, 1952; Boström & Odeblad, 1953; Jorpes, Odeblad & Boström, 1953) found that inorganic ^{35}S-labelled sulphate administered to experimental animals is readily incorporated into an incompletely identified sulphated mucopolysaccharide in the skin and hair follicles and into the mucins of the alimentary tract (as well as into mast cells and the cartilage of growing animals). They also obtained evidence of considerable exchange of sulphate between the various components of the general metabolic pool of sulphated mucopolysaccharides.

It seems conceivable that the administration of a foreign sulphated polysaccharide (such as heparin from a different species, or a heparinoid) might interfere with the metabolism of intrinsic sulphated mucopolysaccharides (possibly by substrate competition), especially where Odeblad and his colleagues found turn-over to be most active (hair follicles and gut), thus producing the transient alopecia and diarrhoea noted clinically. In accounting for the variable distribution of these side-effects in the different series so far reported, dietary and other factors would also have to be considered.

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THE RUBICON: CHANGING VIEWS ON THE RELATIONSHIP OF THROMBOSIS AND BLOOD COAGULATION

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The fact that the title of this symposium is " Blood Coagulation and Thrombosis " is a reassuring sign that one of the most remarkable examples of self-isolation in medicine is coming to an end. If one looks in any of the standard works on haematology written during the last sixty years—Hayem (1889), Ehrlich & Lazarus (1905), Gulland & Goodall (1912), Naegeli (1908, 1931), Whitby & Britton (1935 to 1950), Wintrobe (1942 to 1951), Sturgis (1948), Bernard (1948), Schoen & Tischendorf (1950)—there is scarcely any mention of the mechanism of thrombosis, although there will be much discussion of blood coagulation—the in-vitro conversion of fibrinogen to fibrin by the addition of various extracts which may or may not normally exist in the circulating blood; even Biggs & Macfarlane (1953) in their admirable monograph do not appear to regard thrombosis as a disorder of blood coagulation. On the other hand, textbooks of pathology will give an account of the nature of thrombosis while paying scant attention to the humoral factors that determine the production of a fibrin clot in vivo.

1. The Haematologist's Enforced Interest in Thrombosis

In recent years haematologists have been forced to take an interest in the practical if not the theoretical aspects of thrombosis by the introduction of anticoagulant therapy. It was in 1924 that Mason suggested that heparin might be of value in the treatment of thrombosis, and eleven years later Crafoord (1937) tried it in the prophylaxis of venous thrombosis; the use of dicoumarol for the same purpose was introduced by Butt, Allen & Bollman (1941) and by Bingham, Meyer & Pohle (1941) within two months of the report of its synthesis by Stahmann, Heuber & Link (1941). Best, as the result of his experimental work with Solandt (Solandt & Best, 1938), proposed the use of anticoagulants in the treatment of coronary thrombosis, which in the case of heparin was first adopted by Holten & Lundsteen (1942), and two years later dicoumarol was advocated by Gefter, Kramer & Reinhold (1944). Many clinicians appreciated Best's warning that these drugs are two-edged weapons able to prevent coagulation as well as to inhibit thrombus formation; the more cautious appealed to the coagulation laboratory for assistance, and it was given. However, instead of trying to determine what laboratory method will most reliably give an indication of liability for haemorrhage, the one-stage " prothrombin " time was recommended, even though it was soon shown that it did not measure prothrombin and it was very doubtful if what it did measure (which is in dispute) was the factor concerned with the onset of bleeding. When Biggs & Macfarlane (1953) say, " The tendency to haemorrhage with dicoumarin and tromexan therapy is unpredictable ", and Hunter & Walker (1954) remark, " There is, therefore, beyond certain limits, no hard-and-fast correlation between the results of the Quick test and the tendency to bleed ", is it surprising that Evans (1954), no great enthusiast for this form of therapy, writes:

> Furthermore, coagulability of the blood determined in the laboratory is not an infallible index of blood clotting in the patient. Thus, a number of examples may be met where the prothrombin time is not unduly low and yet the patient bleeds and again where the test told of hypocoagulability while thrombo-embolic incidents were recurring. Such facts supply proof once more that reactions in a test tube in the laboratory differ materially from those shown by the patient in his bed.

It is as though one tried to control the treatment of diabetes mellitus by estimating acetone bodies in the blood and indeed, on occasions in the field of clinical pathology, the coagulationist, tilting his tubes with stop-watch in hand, appears to have inherited the mantle of druidical haematomancy, like the medieval physician who based his prognosis on the appearance of the buffy coat in the bleeding bowl. Such an observation should not be taken too seriously, for studies in blood coagulation have enormously advanced our knowledge of the nature of the haemorrhagic disorders and may well in the future improve their treatment; nevertheless one has the feeling that the stock-in-trade of reagents and reactions to produce a fibrin clot is remote from a thrombus.

2. Blood Coagulation in 1855

Had this symposium appeared a century ago, the subjects of blood coagulation and thrombosis would naturally have been treated together as they were then synonymous. It might be profitable to trace the reason for their segregation and its consequences; we could start by describing an imaginary symposium on blood coagulation in the *British Medical Almanack* for 1855.

George Gulliver (1804–1882) would have written the historical preface. He was surgeon to the Royal Regiment of Horse Guards, but is best remembered for his introduction and notes in the Sydenham Society edition of Hewson's works, which reveal his own considerable contribution as a physiologist; he subsequently became Hunterian Professor of Comparative Anatomy. Gulliver (1846) spoke of the history

of blood coagulation as " a curious chapter in physiological history showing the uncertainty of fame and the slowness of justice; the delusion of hypothesis and the advantage of observation and induction."

It is generally said that Aristotle was one of the first to ascribe the coagulation of blood to the presence of fibrous material, but the humoral nosology was so preponderant in medical thought that little attention was given to the mechanism of clotting until the seventeenth century. Thomas Willis (1659) recognized that serum was coagulable by heat or by acids and was separable from the fibrous clot; this was actually carried out by Malpighi (1666), while Borelli (1681) showed that the serum was divisible into two portions, one coagulable by heat and the other consisting of salt-impregnated water. Ruysch (1707) was the first to produce serum by agitating whole blood with twigs on which the fibrin clotted. In the early part of the eighteenth century there was much confusion with regard to the coagulation of blood, as it was believed that the erythrocytes were essential to the process. Richard Davies, a Fellow of Queens' College, Cambridge, who wrote a most critical essay (Davies, 1760) on the facilities for scientific training at Oxford and Cambridge, set matters to rights by pointing out the basic fractions of blood in its fluid and coagulated state and initiated studies on clot retraction. It is probable that Hunter (1794) gained some of his ideas about blood coagulation from Davies' writings while adding a vitalistic cloak, but there can be no doubt that it is William Hewson (1771) who should be regarded as the first to understand and make understandable the basic facts about blood coagulation, for all that his work was to a large extent ignored until Gulliver (1846) and John Davy (1839) called it to light. Hewson's achievements were: (i) He proved that coagulation of blood is due to coagulation of the plasma, by skimming off the plasma of slowly coagulating blood with a high sedimentation rate, and by showing that the clear plasma clotted. (ii) He ligated a vein so as to include fluid blood within it and, opening it after the corpuscles had subsided, drew off the plasma which clotted. He also showed (iii) that the coagulation of blood drawn from the body cannot be explained as due to loss of heat, arrest of motion or exposure to air; (iv) that coagulation may be inhibited by cold or the addition of neutral salts (Hewson used sodium sulphate) to blood, the process setting in when the conditions are modified; (v) that the walls of living blood vessels exert a remarkable influence on restraining coagulation. After the publication of Hewson's *An experimental inquiry into the properties of the blood* in 1771, there was little progress for 50 years; indeed there was retrogression to the old ideas that the erythrocyte played an essential part in the coagulation process, and some repetition of Hewson's work.

a. Lister's Disposal of Richardson's Ammonia Theory

Continuing our imaginary symposium, it is possible that Dr B. W. Richardson (1828–1896) might have been invited to give a review of the current theories of blood coagulation on which he had been working since 1850; he was about to be awarded the Astley Cooper Prize for his essay on the subject. Richardson is a curious figure in Victorian medicine; he was one of those enthusiasts with multifarious interests, whose fate it was, often to be on the verge of some great achievement, from the full realization of which, perhaps by reason of his versatility, he was nevertheless in some way hindered. He introduced amyl nitrate and chloral into therapy, devised a humane killer for animals, advocated open-air treatment for tuberculosis, founded the *Journal of Public Health* and lectured much on preventive medicine, was a rabid teetotaller, wrote a novel, some plays and poetry, popularized the history of medicine, was an F.R.S. and a K.C.B., an ovate of the Eisteddfod, and gave expert evidence in defence of Dr William Palmer, the Rugeley poisoner. In his review, Richardson would have emphasized that it is agreed that fibrin is essential to coagulation, though whether it exists in circulating blood is in dispute, but that the problem is the cause of the aggregation of fibrin particles upon which coagulation ultimately depends. A low temperature delays coagulation and a high temperature accelerates it, while exclusion of air retards it and exposure accelerates it, but these observations merely reveal favourable and unfavourable conditions for coagulation, and do not attempt to explain the act itself. He would consider the theory of rest, held by older physiologists and recently supported by Dr J. A. Bostock, that by motion the blood is prevented from coagulating and that, when the motion is suspended, the force by which the molecules of fibrin are separated is removed, and by reason of their mutual attraction the first stage of coagulation is initiated. He would recall Hewson's experiments which showed that this was not acceptable as the sole explanation of the phenomenon of coagulation. He would dispose, as Gulliver did, of the Hunterian idea that a vital principle was the cause of coagulation, on the basis of freezing experiments (the modern experiments on refrigeration might make Richardson think again) and he would have little interest in the theory of a nervous force. He would consider a chemical hypothesis much more favourably, pointing out that certain salts can keep the blood fluid and that on their removal the blood coagulates. On the other hand the hypothesis of Carl Schmidt (1850) that fibrin does not exist as such in the circulating blood, but is formed after the blood is drawn, by the transformation of an acid albuminate of soda from a soluble into an insoluble and neutral state, and the subsequent separation of a portion of albumin in the form of fibrin, admits, in Richardson's view, " of no experimental proof whatsoever and is utterly inconsistent with the clearest and best observed facts in nature". Accordingly Richardson (1858) proceeded to chemical experimentation; he satisfied himself that there was an ammonia compound present in the body, which kept the blood fluid, and that on its escape the blood coagulates, and that it might accumulate in the blood, render it too fluid, with consequent disease. He used ammoniated blood for animal transfusions and advocated ammonia therapy for the treatment of fibrinous deposits (thromboses) in the heart and blood vessels. The publication of the Astley Cooper Prize Essay greatly increased Richardson's private practice, as many cases of cardiac disease were sent to him, but, in less than three months after its appearance, Lister (1858) had disproved the ammonia theory by some simple but elegant experiments and in his Croonian Lecture (Lister, 1863) disposed finally of this plausible hypothesis. Lister came to the conclusion that contact with a foreign surface was the initiating mechanism in coagulation, or, as he puts it:

> The real cause of the coagulation of the blood, when shed from the body, is the influence exerted upon it by ordinary matter, the contact of which for a very brief period effects a change in the blood, inducing a mutual reaction between its solid and fluid constituents, in which the corpuscles impart to the liquor sanguinis a disposition to coagulate. This reaction is probably simply chemical in its nature.

b. Buchanan's Concept of Blood Coagulation as an Enzymic Reaction

But we are eight years ahead of our symposium of 1855, which might also have contained an article by Dr Andrew Buchanan (1798–1882) on his experiments on the effects of certain substances on the blood; Buchanan was the first Professor of Physiology at Glasgow University and held the Chair for 47 years, but, as is usually the case, his experimental work was carried out before he reached professorial status. Buchanan (1835, 1845) showed that ascitic or hydrocele fluid would clot if a small amount of blood serum was added to it, and that washed blood clot had the same property and that this could be retained for many months if the clot were kept in alcohol; he observed (1845) that "The power which the washed clot has of coagulating fibrin is not less remarkable than that of rennet in coagulating milk, to which, indeed, it may be aptly compared." Buchanan believed that it was the white corpuscles—not the fibrin—of his washed clot which had coagulant properties and he showed that many tissues—muscle, connective tissue, brain—also possessed coagulant powers; he considered that the active principle was in the cells, and that the fibrin was in solution in the plasma.

Buchanan's work was largely forgotten until Gamgee reprinted it in the *Journal of Physiology* in 1879 (when Buchanan was 81), although Alexander Schmidt of Dorpat, unaware of this earlier work, made similar observations in 1861; however, it was not until 1872 that Schmidt gave up his combination hypothesis and reverted to Buchanan's view that the washed clot or its equivalent acted as an enzyme. Once again we have outstepped our period and have entered what Astrup (1950) has called the first period of experimental work in blood clotting, which he dates from the discovery of fibrinogen by Denis in 1859 to 1904 when Morawitz formulated the classical theory of blood coagulation.

c. Discovery of the Significance of Blood Platelets

Our symposium of 1855 would contain no mention of blood platelets, though they were first seen by Donné in 1842 and then by Zimmermann in 1845, who believed that they were the source of the red blood corpuscles, a view maintained by Hayem (1841–1933) until his death (Hayem, 1877, 1923). Schultze (1865) described them most accurately but was noncommittal as to their nature, as was Osler (1874), who first demonstrated them inside vessels. Riess (1872) suggested that they were breakdown products of leucocytes, but two years later Semmer (1874), working at Dorpat with Schmidt, studied them in amphibians, birds and mammals and showed that they played an essential part in coagulation. This was amplified by Bizzozero (1882), to whom the credit for the recognition of their function is usually given.

3. Development of the Concept of Thrombosis

However, the 1855 symposium would certainly contain an account of fibrinous deposits on the walls of blood vessels and in the heart, because it was this problem and that of phlebitis which had inspired the investigations of many of those who had studied the mechanism of blood coagulation. It is more than probable that this article would have been written by Dr W. S. Kirkes (1823–1864) who was a favourite pupil of Paget's at St Bartholomew's Hospital, London, and who graduated in Berlin in 1846 before qualifying in England. On returning to England he became Medical Registrar and Demonstrator of Morbid Anatomy at St Bartholomew's Hospital and in 1852 published the epoch-making article in which he showed that fibrinous masses formed in the heart could be detached and that they obstructed vessels of distant organs with consequent dysfunction: in other words, the occurrence of arterial emboli with secondary infarction.

It is necessary to retrace our steps a little in order to appreciate why this paper by Kirkes was so important and also perhaps to explain why it is comparatively little known.

a. The Phlebitis and Crasis Theories

From the earliest days of morbid anatomy, clots had been found in the heart at post-mortem examination, and very often these polypi, as they were called, were regarded as the cause of death and certainly formed an obstacle to progress in morphological pathology. Morgagni emphasized that in the majority of instances they were post-mortem phenomena, but by the latter part of the eighteenth century it was recognized that blood could clot in the vessels or cavities of the heart during life. It was Corvisart (1806), the founder of the nineteenth-century Paris School, who set out clearly the features that distinguish an ante-mortem from a post-mortem clot. Thirty years later Jean Cruveilhier (1791–1874) was elected to the first Chair of Pathological Anatomy in Paris and in 1837 enunciated his famous doctrine: "La phlébite domine en quelque sorte la pathologie tout entière". It stemmed from John Hunter's vitalistic views of inflammation, but Cruveilhier was more precise, as he believed that inflammation was characterized by hyperaemia with capillary stasis and a secretion from the capillaries themselves which could be fibrin, pus, or caseous material; inflammation was the commonest of all morbid changes and the property of becoming inflamed indissolubly linked with the living state. Cruveilhier regarded the capillary bed as part of the venous system; and as the immediate seat of inflammation was the capillaries, inflammation of the veins or phlebitis came to occupy the centre of his concept of disease. He distinguished adhesive phlebitis (although the word "thrombosis" was coined by Galen, it did not come into popular usage until about 1856) from suppurative phlebitis, but he failed to recognize the possibility of embolism or the nature of pyaemia, and areas of infarction were called capillary phlebitis, metastases or fibrinous deposits.

One of Cruveilhier's contemporaries in Paris was Gabriel Andral (1797–1876), who wrote the first textbook of haematology and had carried out, with Jules Gavarret, a careful chemical analysis of the blood in various diseases, determining the relative proportions of corpuscles, fibrin, serum and solids; he showed that there was a diminution of fibrin in fevers, in scurvy and after haemorrhage, and an increase in pneumonia. Carl Rokitansky (1804–1878) of Vienna, a magnificent descriptive morbid anatomist, seized on Andral's facts and Cruveilhier's concept of phlebitis to create a theory that all diseases were derived from a primitive affection of the whole blood mass, probably of a chemical nature, which he termed a crasis and which was characterized by a hypothetical variation in the type of fibrin which had a specific affinity for particular localizations; it was a theory susceptible to infinite refinements of classification and interconversion. For example, Rokitansky (1842–46) observed the association between endocarditis and infarction of the distant organs, but he explained this observation "by a poisoned state of the blood consequent on the admixture of the products

of endocarditis with the fluid whereby it acquires an increased tendency to coagulate, which tendency cannot well be maintained in the arteries, owing to the velocity of the current but is brought about within the capillaries on account of the slowness with which the blood there moves and the increased facility which is thus afforded for the morbid material derived from the inflamed heart to exercise its influence on the composition of the blood." It is hardly surprising in such a climate of thought that Dr Hughes, a physician at Guy's Hospital, London, in an excellent article (Hughes, 1839) on fibrinous concretions in the heart, described a case in which a woman with mitral stenosis suddenly developed signs of obstruction of the brachial artery with incipient gangrene; at post-mortem examination she was found to have a thrombus in the left auricle and occlusion of the brachial artery by clot; " The fair and natural presumption ", writes Dr Hughes, " is, that both masses of fibrin assumed the solid form about the same time ".

b. Virchow's Contributions to Blood Coagulation and Thrombosis

Rudolf Virchow (1821–1902) graduated in 1843, and his chief, Froriep, suggested that he should investigate the soundness of Cruveilhier's concept of phlebitis; being of a critical rather than a constructive turn of mind, this was a problem after his own heart. He first of all studied blood coagulation and the nature of the fibrin clot from a chemical point of view; he decided that there was only one type of fibrin, which could not be detected in the blood stream (or tissues) but only by the act of coagulation, and that most of the fibrin in the body was fibrinogen (Virchow coined the term) which was formed in the lymphatic and connective tissue, and was activated or converted into fibrin by the presence of oxygen. Virchow then went on to study obstruction of the pulmonary artery and, observing the frequent association of infected venous thrombi with pulmonary abscesses, introduced the revolutionary doctrine of embolism in his first paper on the subject, in 1846 (Virchow, 1846a); it was true, as Virchow pointed out, that Paget had observed the association some years before, but as Paget (1901) wrote many years later:

> In [my] records I find long descriptions of the morbid changes in different forms of embolism, but I wholly overlooked and missed their meaning till it was discovered by Virchow and my own pupil Kirkes, and by others more observant than myself. I saw all these things; they accurately observed them; they studied the associated facts; and embolism, which had been destroying healths and lives for ages in secret, was at last discovered.

In the same year Virchow (1846b) published a second paper in which he described experiments on dogs and studies of further cases of pulmonary embolism: however, all these observations were on venous thrombi and embolism, although in the second paper he mentioned that he had observed analogous changes in arteries and that in a future paper he would show that these were also due to transport of clot in the circulation and not due to local blood clotting. However, this third paper was not published until later and a paper on acute inflammation of arteries (Virchow, 1847) deals largely with arterial thrombosis. The full account of Virchow's ideas on thrombosis and embolism was first published in 1856 as a chapter in his magnificent *Gesammelte Abhandlungen zur wissenschaftlichen Medicin*; he mentions Kirkes' paper of 1852 but suggests that really he himself had described it all before, which is not quite true; but then Virchow was not very willing to agree that anyone other than himself could have sound ideas. On the other hand it must be confessed that Kirkes does not mention Virchow's observations on venous thrombi, which it is inconceivable that he had not heard about while he was in Berlin.

Virchow's introduction of the concepts of thrombosis and embolism were of the first importance from the point of view of general pathology, yet their mechanistic simplicity deflected attention, as far as clinicians and pathologists were concerned, from the process of coagulation which initiated the thrombus; so it was that a segregation of interests began, and this became more marked as the problems of blood coagulation became the prerogative of the physiologists who studied, in test-tubes, the formation of fibrin clots from fractionated plasma.

4. Recognition of the Haemorrhagic Disorders in 1855

It is a little doubtful if our symposium of 1855 would have included an account of the haemorrhagic disorders, as it was generally felt at that time that purpura was a skin disease related to naevi and that the haemorrhagic diathesis or idiosyncrasy (as haemophilia was then called) was a disorder of the blood vessels. However, other ideas about these conditions were evolving, and as Henry Ancell of the Grosvenor Place School of Medicine, London, had included both conditions in the excellent course of lectures which he gave in 1840 on the physiology and pathology of the blood, it is not taking too much licence to imagine that he might have written a review of these conditions 15 years later.

a. The Purpuras

Although purpuric conditions have been recognized from the earliest times, the clinical type sometimes associated with the name of Werlhof was described by the Hanoverian physician in 1735, while the elder Heberden gave an admirable account of the anaphylactoid form in 1802, whereas Schönlein's description appeared in 1837 and Henoch's in 1868. However, the first suggestion that it was a disorder of blood coagulation is to be found in an account by Andrew Duncan (1773–1832), Professor of Materia Medica at Edinburgh, published in 1822. He described a fatal case of purpura in a boy aged 15, and as the post-mortem revealed petechiae everywhere, he questions the propriety of considering it as a cutaneous disease and suggests that it might be due to " Increased tenuity of the blood, allowing it to escape from the superficial extremities of the minute arteries." Furthermore, he observed that the coagulation of the blood was not firm: " No serum was separated. The coagulum had the appearance of jelly, being tremulous and transparent, in itself almost colourless, but with a pink tinge, from the red globules which had sunk to the bottom seen through it."

In the same number of the *Edinburgh Medical and Surgical Journal*, George Johnston (1822) described another case of purpura in which there was no clot retraction—" The blood drawn in the morning has not separated into serum and crassamentum. It possessed little consistence or tenacity; but there are traces of coagulable lymph diffused through it "; it was the submission of Johnston's case report that had stimulated Duncan, who was the editor, to describe his case with his more significant conclusions. In the following year William Stoker (1773–1848), Professor of Medicine in the short-lived Eccles Street School in Dublin, published in 1823 a book of

Pathological observations on dropsy, purpura . . . and particularly on the morbid changes of the blood, and their influence on the production and course of these diseases, in which he determined the coagulation time and the " flowing time " and noted that the latter was prolonged in purpura. However, it was not until 1883 that Krauss reported that the platelets were reduced in purpura, while Hayem in 1895 was the first to count the platelets in a case and also reported the deficiency of clot retraction. After Stoker's observation on the flowing time of the blood in purpura there were casual observations on the prolonged bleeding time in purpura, but the matter was not studied critically until Duke (1910) emphasized the distinction between the bleeding and coagulation times.

b. Haemophilia

With regard to the haemorrhagic diathesis (Schönlein is usually credited with inventing the term haemophilia in 1837, but actually his suggestion was haemorraphilia, whereas haemophilia was coined by Hopff (1828); but it did not come into general usage until after the publication of Grandidier's monograph in 1855), Ancell in 1839–40 describes it as:

> . . . a remarkable state of the blood and sanguiferous system, by no means of very uncommon occurrence even in this country. It is hereditary, and has been observed to affect the males of a family most frequently, although it may be transmitted by the mother.

It had been noted that not only was there persistent oozing from the injuries, but the blood failed to clot and James Wardrop (1835), in his monograph on blood letting, collected together a number of cases and considered that the disease was due to a deficiency in the coagulating powers of the blood, whereas Liston (1838–39) in a clinical lecture on a case stated:

> . . . it was equally necessary in such cases to examine into the state of the blood as well as the condition of the vessels, as much, no doubt, depended on that . . . The blood (which had been examined through a microscope), although the man looked so strong and healthy, differed from the usual character of this fluid, and seemed to be deficient of fibrine.

Even more remarkable was the observation made by Heinrich Rieken (1829), in his admirable monograph on a haemophilic family at Nohfelden, that the addition of normal serum caused haemophilic blood to clot. Ancell assisted Samuel Lane (1840–41) in the first case of haemophilia to be successfully treated by transfusion; Lane operated on a boy aged 11 for squint, and when bleeding persisted, he was informed that the boy had been in St George's Hospital, London, on a number of occasions for intractable haemorrhage from minor injuries and for arthritis. The bleeding continued, with intermissions achieved by local pressure, for six days and then Lane made up his mind not to allow the boy to die without giving him the chance afforded by transfusion. He took advice on the technique from Dr George Blundell and, using Dr Blundell's equipment, five and a half ounces[1] of blood, taken from a stout healthy young woman, were injected into the vessels of the boy.

> The beneficial effects, at least to any marked extent, although the pulse immediately returned at the wrist, were not instantaneous; but, in the course of an hour or two, the boy sat up in his bed, and drank a glass of wine and water from his own hand; and the contrast between life and death could scarcely be greater than his state at this moment compared with that of a few hours before. There was no recurrence of the bleeding from the eye. The wound at the bend of arm healed in about ten days.

[1] 1 oz. = 28 g.—Ed.

Ancell concludes his lectures on the blood with these words:

> . . . I think it has been rendered abundantly manifest, that the doctrine of diseases must ever be imperfect, and their treatment empyrical, until we are better acquainted with the pathology of the blood, and that this is a fertile field, only requiring cultivation.

5. The Separation of Blood Coagulation from Thrombosis

I have tried to show that a hundred years ago the field of blood coagulation had been cultivated in close relationship to the problems of thrombosis and haemostasis; it now remains to sketch the dividing of the ways, and in a sense it is the story of specialism. In 1855, observations and experiments on thrombosis and coagulation were being performed by physicians and surgeons who were inquisitive and wanted to find out the answers to their practical clinical problems and also to problems of general biology, but after that the experiments were carried out by pathologists, physiologists, haematologists, coagulationists—and so the streams divide.

a. Thrombosis as a Problem in Tissue Pathology

After Virchow's clarification of the nature of thrombosis and embolism, we have the work of Cohnheim, Zahn, Senftleben, Baumgarten, von Recklinghausen, Eberth and Schimmelbusch crystallized in the great review by Welch (1899) in Allbutt's *System of medicine*, which was great not only because it marshalled critically all the work of the previous half century, but because it was written by one who had made considerable contributions to the subject. For the next quarter century we have Aschoff's lecture, delivered in the USA in 1924, in which he says:

> But all histological research . . . speaks for the view that in human beings the occurrence of fibrin coagulation is not the first stage of thrombosis, but that important changes in the morphological blood constituents precede it. These last-named changes must be explained before the mechanism of thrombosis can be understood.

b. The Academic Attitude to Blood Coagulation

To turn to blood coagulation: after the work of Buchanan, Richardson and Lister, investigations proceeded in the capable hands of physiologists such as Schmidt and Hammarsten, leading to the evolution of the classical theory of blood coagulation, until an imaginative Irishman disturbed the academic peace and tried to bring the theories back to the problems of thrombosis and haemorrhage. Shortly after qualification, Almroth Wright went to Leipzig to work under Cohnheim, Weigert and Ludwig and there met Leonard Wooldridge, a laboratory assistant from Guy's Hospital, London, who was investigating blood coagulation and trying to get a medical qualification at the same time; to appraise Wooldridge's (1893) work in the light of modern knowledge would be beyond my abilities, but it is important to remember that Wooldridge was the first in modern times to induce intravascular coagulation (it is doubtful if it was a true thrombosis) by the injection of tissue extracts into animals. Wright became interested in blood coagulation, and when he was working at the Army Medical School, Netley, England, he began to study haemophilia and, taking hold of Arthus & Pagès' (1890) observation that calcium was an essential factor in normal blood coagulation, he came to the conclusion that

there was calcium deficiency in haemophilia which could be corrected by administering this metal and that the abnormality and its correction could be displayed by means of the capillary tube coagulometer which he had devised. He went further and suggested that in thrombosis there was a shortened clotting time and that this might be due to too much calcium and that this could be corrected by the administration of citric acid; furthermore, urticaria might be regarded as serous haemorrhages, and again the clotting tests were prolonged so that calcium treatment should be effective. His hypothesis and observations were unsound, but calcium therapy is still exhibited for chilblains if not for haemorrhagic disorders, just as Rokitansky's crasis survives in the layman's blood poisoning.

c. The Effect of Almroth Wright's Invasion

Wright (1893) was the first to suggest a specific defect in haemophilic blood and, though he was soon disproved, yet in disproving him, Sahli, Nolf, Morawitz, and others found that there was something odd about haemophilic blood. As Biggs & Macfarlane (1953) say, " In the course of these intensive investigations almost every factor known to be involved in the clotting process was at one time supposed to be abnormal in haemophilia, but in each instance these suppositions were proved baseless. Always it was found that the defect seemed to lie just beyond the widening scope of existing knowledge "; and as factor after factor was revealed, it has taken investigators farther and farther away from the two fundamental questions—why does normal blood stay fluid in the vessels, and what are the conditions that induce thrombosis?

6. Morphological Variants of the Fibrin Clot

There are a very large number of morphological variants associated with the conversion of fibrinogen into fibrin, and there is no a priori justification for assuming that the mechanism in each case is identical. First there is thrombosis—" a solid mass or plug formed in the living heart or vessels from constituents of the blood " (Welch, 1899), and we may distinguish thrombi occurring in arteries, veins or capillaries, and thrombi predominantly formed of fibrin or with a varying admixture of cellular elements. Then there is the experimental or therapeutic induction of a fibrin coagulum in the vessels that usually cannot be regarded as a thrombus, in that it is ordinarily achieved by the introduction of some substance into the blood stream; the coagulation of extravasated blood in haemostasis, whether from artery, vein or capillary, is another variant, and this may be spontaneous or associated with the application of an adjuvant. Lastly there are examples of non-vital blood coagulation—the intravascular post-mortem clot, the coagulation of whole blood or plasma in a test-tube which is made of a wettable or non-wettable substance, and the production of a fibrin clot by the admixture of various reagents. To consider in detail each of these varieties of fibrin formation would be a herculean task; indeed it is doubtful if at the present time there is enough information to justify such a review, but it might be interesting to consider briefly the general biology of coagulation and attempt to relate it to haemostasis which can be regarded as the form of blood coagulation that is most nearly physiological, at any rate in the corpus haemorrhagicum of the ovary, the menstruating endometrium, and that following placental separation.

7. Evolution of the Surface Precipitation Reaction

It is generally held that alteration in protoplasmic viscosity is a determining factor in very many biological processes and is the basic response to stimulation; it has also been shown that the mechanism of this sol–gel reversible reaction is analogous to that occurring in the fibrinogen–fibrin transformation and is controlled by similar influences.

Amoeboid movement is probably due to a transformation of gel to sol at the advancing tip of a pseudopod, while there is an increased viscosity allowing contraction of the cortex or plasma gel at the posterior end of the animal; calcium is essential for this type of movement to take place. In muscular contraction the fundamental change is a conversion of the actomyosin to a gel state mediated through adenosine triphosphoric acid (ATP); not only does calcium play a part in this process but muscular contraction can be induced by the presence of thrombin and inhibited by heparin, although neither of these substances has any known relation to ATP.

In cell division, it has been shown by Heilbrunn (1952) and others that the appearance of the mitotic spindle is preceded by a sharp increase in protoplasmic viscosity, and that those agents which induce artificial parthenogenesis all induce protoplasmic gelation; in many of them it can be shown that their immediate effect is a release of calcium from combination with protein or lipoprotein. In the frog, artificial parthenogenesis is made possible only by introducing blood serum or tissue extracts into the cell, and heparin is able to inhibit mitosis in a large range of cell types (Heilbrunn & Wilson, 1949), while heparin or heparin-like substances have been detected in the eggs of certain species.

In normal fertilization when a spermatozoon attaches itself to the egg surface, there is the formation of a fertilization membrane which becomes coagulated, while the cortex contracts; this process can take place only in the presence of calcium and is inhibited by heparin: however, interestingly enough, the addition of ATP allows these processes to proceed normally (Rummström & Kriszat, 1950).

When a unicellular organism is injured—and indeed the phenomenon is also observed in tissue cells of multicellular organisms—the fluid contents do not disperse throughout the surrounding medium, but, as the protoplasm starts to flow out, a new film or membrane forms at the border of the emerging droplet; this has been called the " surface precipitation reaction " and was at one time thought to be a surface tension phenomenon. However, it has been shown that it depends on the presence of calcium in the surrounding medium or on an extract of damaged cells—the source of thromboplastin. It has, moreover, been suggested that, in injury, the stimulus to cell division—which is the essential mechanism of repair—is the release of such substances from the damaged cells, but our knowledge of the biochemistry of such processes is as inadequate as it is in blood coagulation.

The evolution and comparative physiology of these processes, which range from the mechanism for the retention of tissue fluids to haemostasis, remains to be studied. Loeb (1928) has investigated the process in a number of species, and his findings in the king crab, *Limulus polyphemus*, a living fossil from the upper carboniferous period, are of considerable interest. In this arthropod, fibrinogen is lacking, and it has only one type of cell—the amoebocyte—in its circulating fluid. If threads are inserted into its fluid cavities, the amoebocytes

attach themselves to the threads, round off, stick together and form a clot-like material which gradually retracts; furthermore, this pseudo-coagulation is reversible. A similar process can be induced in vitro, but, if the tissue fluid is collected on a non-wettable surface, the amoebocytes sink to the bottom of the vessel and only agglutinate one to another, forming a thin tissue-like layer of cells. In the sponges analogous processes occur, although the presence of several cell types in the fluid makes interpretation more complex, while Hardy (1892) observed in *Gammarus*, one of the sand fleas, coagulation-promoting explosive corpuscles as well as pseudopodial extrusions of the type seen in *Limulus*.

The occurrence of extracellular coagula, as well as of cell agglutination, is first observed in certain of the decapods such as the lobster, in which substances can be extracted from the agglutinated corpuscles or damaged tissue cells which induce coagulation of the blood, and calcium has an influence on the process. In vertebrates the process appears to become more complicated the more it is studied, and as one evolves from the nucleated spindle cells of the birds to the blood platelets of mammals; there is little doubt that much profit could be gained if the congenital abnormalities of blood coagulation were looked at from the Garrodian point of view as has been attempted recently by van Creveld & Mochtar (1954).

8. Signs of a Revived Interest in the Clotting of Whole Blood

If there is a justification for regarding haemostasis as an evolved form of the " surface precipitation reaction " of the unicellular organism and related to the general phenomenon of protoplasmic viscosity, then it is of paramount importance that an investigation of the process of thrombus formation should be approached biologically from the interrelationship between the tissues and the fluid blood and not by the esoteric study of the unnatural production of a fibrin clot in a test-tube. On the other hand it is clear that in-vitro studies are profitable from the physico-chemical point of view, and recent applications of the methods of protein chemistry are laying bare the kinetics and basic chemical reactions in the conversion of fibrinogen to fibrin. There have already been skirmishes across the frontier: Macfarlane, Jaques and Roskam have always thought of blood coagulation in relation to haemostasis; recently the study of the clotting of whole blood in the test-tube has more than confirmed the old idea that shed blood produces its own thromboplastin and has revived an interest in the initial stages of blood coagulation: studies on blood platelets and the in-vivo effects of anti-coagulants by Helen Payling Wright and others is another attack; work on serotonin awaits further integration and then the haemostatic mechanism should begin to be clearer. Sawyer & Pate's (1953) work on the influence of the potential difference of the vessel wall on thrombosis, and Samuels & Webster's (1952) display of the interrelationship between heparin, a cement substance and the local site of thrombus formation, is another approach and one which might give the clue to the integration of the heparin-clearing reaction and thrombosis. However, these observations, valuable as they are, are only border raids, and an integrated attack on the problem in its broadest aspects would certainly be most rewarding.

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Book Reviews

The Distribution of the Human Blood Groups

A. E. Mourant. Oxford : Blackwell Scientific Publications, 1954. xxi + 438 pages; illustrations. 23 × 15 cm. £2 2s.

It is just 25 years ago that Dr and Mrs Hirszfeld published their observation that the ABO blood groups were distributed unequally among the many populations mingling in Salonika during the First World War. When W. C. Boyd's compilation of ABO blood-group surveys appeared in 1938 it had become clear that here was a most useful anthropological tool. With the increasing number of blood-group systems known, and the spread of surveys all over the world, a new classification of mankind is emerging, which may justly take its place beside those based on skull shape, skin colour or hair texture, on cultural traditions, structure of language and religious and sociological systems. It has been possible to gain some information about this development from Wiener's *Blood groups and transfusion* (1943) and Boyd's *Genetics and the races of man* (1950). However, the data accumulated to such an extent that it seemed too much to be hoped for to see them sifted and co-ordinated in a single book. The demands such a work would make on the combination of technical knowledge of blood grouping with a true understanding of human genetics and of anthropology seemed too great. We must be grateful that after all there exist, even in the 20th century, such encyclopaedic scholars as Dr Mourant. The Director of the Medical Research Council's Blood Group Reference Laboratory is not only an authority on blood groups—as one might indeed expect—but also a recognized geneticist and an anthropologist of note.

The first half of the book discusses the application of Mendelian characteristics to anthropology in general and to some dozen human blood-group systems in particular, as well as describing their genetics and serology. A special chapter deals with characters which are similar to blood groups in being both common and genetically simple: for instance the recessive characters of red hair and blue eyes, the ability to taste phenylthiocarbamide and the hereditary abnormalities of haemoglobin production (thalassaemia and sickle-cell trait). There follow surveys of gene distribution in northern and central Europe, the Mediterranean area, Africa south of the Sahara Desert, Asia, Indonesia and Australasia, and aboriginal America. Migrant and hybrid populations are considered by themselves and special chapters are set aside for blood grouping of bones and mummies (" blood-group archaeology ") and of animals. All these are surveyed together in a chapter entitled " An attempt at a synthesis ", which is a particularly valuable part of the book. The relatively uniform frequencies of most of the blood-group systems over large areas imply a long-term stability, yet—as with all genes—the blood groups must be subject to natural selection, and the comparison between variations of different systems—some uniform over large areas, some varying widely—leads to the assumption that the latter are more intensively selected by environment. Thus the frequency of the rhesus blood groups, subject as they must be to selection by haemolytic disease of the rhesus-negative new-born, is nevertheless much more alike over whole continents than that of the ABO blood groups. The recent discovery of a connexion between cancer of the stomach and blood group A by Aird, Bentall and Fraser Roberts has shown that such selection does indeed occur.

The first half of the book closes with technical chapters on blood-grouping methods and on the calculation of blood-group frequencies. The second half of the book contains a bibliography comprising 1716 entries, nine maps of blood-group distribution, 39 blood-group frequency tables and an exhaustive table of frequencies of the sickle-cell trait. They are followed by three indexes, two to the bibliography—topographical and zoological—, and one to the text. There are thus two books in one, the first half stimulating for the expert as well as for those generally interested, and the second half a valuable book of reference for the research worker in this field, though certainly the maps will be equally fascinating for all.

H. Lehmann

Blood Groups in Man

R. R. Race & Ruth Sanger. 2nd ed. Oxford: Blackwell Scientific Publications, 1954. xvi + 400 pages; 34 figures. 22 × 14 cm. £1 10s.

When the 1st edition of *Blood groups in man* appeared in 1950, it at once established itself as the standard work on the human blood groups, a position which it has since held without a serious rival. Since its appearance, however, research in the subject has been very active and fruitful. The results have not been revolutionary, as was the discovery of the Rh blood groups, but they have substantially affected the genetical situation, especially as it concerns the Rh and MNS blood-group systems. One completely new major blood-group system has been discovered. Serologically, new discoveries have been of less importance, while the clinical situation remains dominated by the importance of immunization of Rh-negative individuals by the Rh or D factor.

The 2nd edition of *Blood groups in man* sensitively and fully reflects the new discoveries and developments. Not surprisingly, it has been necessary to increase the number of pages by about 100. In these days of increasing prices it is most satisfactory to find that the publishers have been able to increase the size without adding to the price.

Following two introductory chapters, each of the nine major blood-group systems is described in one or more chapters. There is one chapter on certain less clearly defined or less important blood groups, then six chapters on special aspects of the subject, including methods of testing, problems of parentage and identity, and genetic linkage. The book thus remains a very complete and readable guide to all aspects of the blood groups, but with special emphasis on genetics and on the discoveries of the past 15 years. It does not set out to be a laboratory manual, but the chapter on methods of testing will be of great value to those who already have a good grounding in the subject.

Those who already know the 1st edition [reviewed in *British Medical Bulletin*, 1951, Vol. 7, p. 358] will be mainly interested in the changes and additions. Under the heading of the ABO groups there is a judicious account of the antigens that have been named C, H and O, concerning all of which there has been much controversy.

Much more interesting are the accounts of numerous discoveries in the Rh system, leading up to a penetrating study of the theory of close linkage, now no longer an isolated and questionable blood-group hypothesis, but something which more and more is being discovered in all branches of genetics. To the Kidd blood groups is devoted a whole new chapter. The new chapter on linkage not only records the first one, or perhaps two, examples of autosomal linkage to be established for man (between Rh and elliptocytosis, and between the Lewis and Lutheran blood groups), but it summarizes much inconclusive work and many negative results obtained in the past, and so clears the field for the many more positive results which are now to be expected.

The references, indexing, printing and paper are admirable; errors are exceedingly few and there is very little that invites adverse criticism. The theories, and especially Grubb's theory, of the relation between the Le^a and Le^b antigens ought perhaps to have been treated more fully; it is questionable whether the 18 pages devoted to Table 105 are justified, but it would be interesting to know whether, in fact, forensic serologists make practical use of it in paternity examinations; in German references repeated from the 1st edition many nouns lack initial capitals, but the spelling of new references conforms to convention.

This book should be in every reference library which includes pathology and genetics; in every laboratory where blood grouping is done; and, in view of its relatively low price, in the hands of every individual serologist and geneticist. Many other scientists of catholic tastes will also find it of great interest. The changes and additions introduced into the 2nd edition are such that most owners of the 1st edition will find it worth while to obtain the 2nd.

A. E. Mourant

The Separation of Protein Fractions from Human Plasma with Ether

R. A. Kekwick & Margaret E. Mackay. London: Her Majesty's Stationery Office, 1954. (Medical Research Council Special Report Series No. 286.) 75 pages; 5 plates; 17 figures. 24 × 15 cm. 6s.

In this Special Report the authors describe with adequate technical detail a system for the fractionation of human plasma along the lines developed by Cohn and his school in the USA, but have substituted with advantage the use of ethyl ether for ethanol as the fractionating agent. Otherwise the method is similar to Cohn's, the various fractions being obtained by varying the pH, ionic strength, temperature, and ether concentration. A feature of note has been the use of a rigid aseptic technique throughout.

In assessing the final products of any attempt at fractionation of a complex system of biopolymers, the final criteria of purity should be functional. Too rigid adherence to the older classical concepts of "chemical purity" or molecular individuality when proteins are being dealt with has led in the past to the "pure substance" frequently being of less value medically than the original starting material. Kekwick and Mackay appear to have successfully avoided this pitfall. They have developed a relatively simple method of obtaining γ-globulin, fibrinogen, albumin and thrombin in high concentration and yet of marked biological activity. Their albumin fraction is completely homogeneous as judged by electrophoretic and ultracentrifugal analyses. Injection of the albumin fraction alone does not give rise to homologous serum jaundice. This, together with its low viscosity relative to concentrated whole plasma, should lead to its increasing clinical use. The γ-globulin is obtained with a similar degree of physico-chemical purity. The clottable nitrogen in these authors' purest fibrinogen represents more than 80 % of the total nitrogen in the fraction. It has a biological life exceeding 300 hours at 37° C. As usual this fraction shows the activity associated with the so-called antihaemophilic globulin (AHG), but does this represent contamination of the fibrinogen or is the antihaemophilic activity associated with a labile prosthetic group on the fibrinogen molecule? This relatively pure fibrinogen should be of value in the further study of this question. The authors note that the degree of antihaemophilic activity is too variable to justify its use clinically. The thrombin fraction, although of adequate activity for clinical use, is by no means pure. It represents a convenient concentrate for further research. There are most useful chapters on the experimental basis of the fractionation procedure, the reagents employed, and their analytical methods and assays, with full descriptions of the equipment employed. The work concludes with a short and cautious survey of the clinical applications of the plasma fractions, and a brief account by N. H. Martin of his clinical experiences with the albumin fraction. There is a most valuable list of references. This is a monograph that should be in the possession of all who may be interested in plasma fractions and methods of protein fractionation.

J. F. Wilkinson

Lectures on the Scientific Basis of Medicine. Volume II: 1952–53

British Postgraduate Medical Federation, University of London. London: Athlone Press, 1954. xi + 380 pages; 29 plates; figures. 22 × 14 cm. £1 15s.

The British Postgraduate Medical Federation of the University of London each year arranges a series of lectures which are eventually collected in book form. An effort is made to cover those aspects of medicine in which significant advances have been made and which have some practical bearing on clinical problems. The lecturer invited to deal with each selected topic is a person actively engaged in furthering advance in it by original research. The second series of lectures to be published fully maintains the high standard set by the first.

P. L. Mollison contributes a section on the life-span of red blood cells. The Ashby technique, which enables the survival of group-O red cells transfused to patients of blood group A to be followed, continues to be the most widely used method. Techniques using ^{15}N-labelled glycine, which is incorporated in newly formed haemoglobin, seem more useful in studying haemoglobin metabolism than in giving precise estimation of the life-span of red cells. Methods using radioactive iron have the disadvantage of radiation hazard, for radioactive iron has a long half-life. This method can be used only in a subject in whom re-utilization of iron is minimal. The technique is therefore safely applied only to those with haemochromatosis or aplastic anaemia. Diminished red-cell survival is characteristic of acquired haemolytic anaemia. Red cells from patients with spherocytosis survive poorly in a normal recipient. One wishes that the clinical applications had been amplified: the diminished survival in renal failure is mentioned but that in carcinomatosis, the leukaemias, Hodgkin's disease and liver failure is barely touched upon.

J. C. White writes a full and excellent account of human haemoglobins. The evidence for the existence of foetal and adult human haemoglobins and for three genetically controlled abnormal human haemoglobins is discussed and related to the clinical findings in various anaemic conditions. The advantages of precise physicochemical methods for their identification are stressed. In a large bibliography Dr White has covered the world literature. This is the best review of the subject available.

P. C. C. Garnham describes the life history of the malaria parasite, and includes the cycle in the blood and newer concepts of the development of the sporozoite in the parenchymal cell of the human liver.

A. S. Parkes describes his observations on the viability of spermatozoa after freezing. This has since been extended to include the preservation of red blood cells at low temperatures.

The reviewer has drawn particular attention to the chapters dealing with haematological problems, but there are also admirable contributions on physiological topics by W. S. Feldberg, A. St G. Huggett, W. D. M. Paton and W. K. Stewart. More-biochemical contributions are made by C. E. Dent, G. F. Marrian, A. Neuberger and R. H. S. Thompson.

This is a book that will prove valuable to workers of many different disciplines. It is well produced and illustrated and the price is reasonable. It is a credit to British medical research and can be wholeheartedly recommended.

Sheila Sherlock

Lectures on General Pathology

Edited by *Howard Florey.* London: Lloyd-Luke (Medical Books) Ltd., 1954. xiii + 733 pages; illustrations. 24 × 16 cm. £3 3s.

The lectures in this book are drawn from the 16-week course in general pathology and bacteriology, which is held primarily for those who have already read the Honour School of Physiology at Oxford. They do not form a complete course in these subjects, but aim rather "to treat of some of the fundamental changes that take place in the body in response to injury, using the word in a broad sense, and to discuss some present-day views about the nature and causes of such changes." Sir Howard Florey and his team have succeeded admirably in their aim, and there is no doubt that any student with the scientific knowledge to understand the lectures and the mental capacity to digest so concentrated a feast will have a much profounder understanding of medicine than his fellows. Practising pathologists will perhaps benefit even more from the broad outlook and magnificent correlations of these lectures.

The approach throughout is that of the scholar, aware of the historical roots of his subject as well as of its most recent ramifications. The opening lecture on the history and scope of pathology sets a standard of critical appraisement that is rare in medical history but that is nevertheless maintained throughout the book.

The individual lectures are of about uniform excellence, but it is perhaps not invidious to say that those of Sir Howard Florey show to a particular degree the luminosity that he has shed over the whole book. The haematologist will naturally turn first to Dr R. G. Macfarlane's chapters on the reactions of the blood to injury, where even the subject of coagulation is made to seem simple. The less dazzling but more solid performance of these lectures is to relate blood changes clearly to the reactions of the whole body.

The publishers have produced a book worthy of its contents, with an unusually high quality of paper and standard of reproduction.

Martin Hynes

British Pharmaceutical Codex 1954

Pharmaceutical Society of Great Britain. London: Pharmaceutical Press, 1954. xxxii + 1340 pages. 23 × 15 cm. £3 3s.

In 1903 the Pharmaceutical Society of Great Britain resolved to produce a book of reference for those engaged in prescribing or dispensing medicines, and this book appeared in 1907 as the first *British Pharmaceutical Codex*. It contained information on all drugs and medicinal preparations commonly used in Great Britain, France, Germany and the United States of America. At intervals, new editions were necessary to keep the book up to date; at first they were issued every ten years, but later (as medicine advanced more rapidly) every five years. The present edition thus supersedes the edition issued in 1949.

In a way this book is a supplement to the *British Pharmacopoeia*. The latter gives the official legal descriptions of the well-recognized drugs, and lays down standards as to their purity and similar attributes. The *Codex* gives much other useful (but non-official) information about the prescribing and properties of these substances; and in addition it gives authoritative accounts of many new drugs which have been introduced during the last few decades but which have not yet been accorded formal recognition by the *Pharmacopoeia*. The number of such new compounds is now so large that selection is difficult; but the inclusion of such a drug in the *Codex* is rightly regarded as an indication that it is of true value. However, the real criterion in judging which drugs should be included in the *Codex* is the extent to which they are in actual use. Accordingly many substances have been included because they are still employed for traditional reasons, but the descriptive monographs indicate that their clinical value is doubtful; and in a few cases (to avoid difficulties that would otherwise occur in connexion with the Dangerous Drugs Regulations), such as in the monographs for preparations of diamorphine hydrochloride and similar substances, they have been retained in the *Codex* with a warning that the drug should be used with great caution and that other less dangerous cough depressants should be given preference if at all possible.

The *Codex* is divided into six parts and there are thirteen appendices. Part I contains over 800 pages of general monographs, relating to almost as many medicinal substances. Each monograph gives the chemical constitution and properties, the solubility, and the standard limits of purity within which an acceptable specimen must lie; then there is a brief account of its therapeutic properties (including the possible toxic effects) and the dosage and methods of administration, together with details about sterilization and the preparations which are officially available. It is noteworthy that the names of the substances are now given first in English, although the synonyms usually include the Latin equivalent. Part II (which occupies 50 pages) deals with antisera, vaccines and related substances, about 50 being mentioned altogether. Part III (12 pages) describes preparations of human blood, such as whole blood, fibrinogen, fibrin foam, thrombin, concentrated red-blood corpuscles, plasma and serum. Part IV (2 pages) deals with surgical ligatures and sutures, and Part V (40 pages) with surgical dressings. Part VI (250 pages) is the Formulary giving methods of preparation and specifications for the various extracts, infusions, ointments, and other pharmaceutical preparations. The 13 appendices occupy 60 pages, giving atomic weights, etc., details of reagents, and methods of carrying out the most important tests to check composition and sterility.

It would be presumptuous to recommend such a volume, for the *Codex* is its own recommendation. One can only congratulate all those who have taken part in its preparation for having compiled such an enormous amount of accurate information which will be indispensable to anyone concerned with the production, handling or dispensing of drugs and medicinal substances.

F. Hawking

Proceedings of the First World Conference on Medical Education, London, 1953, held under the Auspices of the World Medical Association

World Medical Association. London: Cumberlege, 1954. xvi + 804 pages; illustrations. 25 × 16 cm. £3

Medical education can always be relied upon to evoke a vigorous discussion, and an international medical conference can be relied upon to be a success because of the opportunities it affords for experts from many countries to meet each other. The First World Conference on Medical Education, held in London in August 1953, did more than fulfil these expectations, as the volume of Proceedings shows.

Each of the four main subjects discussed—the selection of students and teachers, the content of the undergraduate medical curriculum, the techniques of teaching and the need for the education of medical men in preventive medicine—produced criticism of the present practices from all parts of the world, but two main themes can be discerned throughout: firstly that a human being, in health or illness, must be comprehended as a whole, and secondly that training for a career, even that of a general practitioner, should not be the purpose of undergraduate education. There are as many statements of the aim of medical undergraduate education as there are writers and speakers on the subject, but these are usually to the effect that the purpose should be to prepare the student to make good use of his experiences throughout his professional life so that he may grow in knowledge and wisdom. Sir Richard Livingstone told the Conference that the aim of all education is to " train our youth to desire, recognize, and pursue the first-rate ". Specialization is necessary for the advancement of knowledge and for the application of new knowledge to the practice of clinical medicine, but the comprehension of man as a whole in health and in his reactions to disease does not require instruction by clinical specialists. There was general agreement at the Conference that the medical curriculum needs to be examined anew in the light of this criticism. It is frequently pointed out that the great majority of graduates find their careers in general and family practice and that the curriculum should be designed with this in view. It was salutary to find it stated that general practice is one type of medical career among many and that the curriculum should be essentially educational and not vocational even if the majority of graduates find their vocation in one particular field. Sir Lionel Whitby drew attention to the increasing influence of the basic medical sciences on the practice of medicine and to the need for more—not less—education of the undergraduate in these subjects. The outstanding plea in the section on the medical curriculum was for greater co-ordination of the basic sciences with clinical studies.

Everyone who has an interest in medicine and in the education of medical men and women will find much with which he can agree, and something with which he will disagree, in this record of the Proceedings; he will be stimulated to think again on the many and varied problems of medical education; and from the experiences collected here from all over the world he may find something that will influence for the better his approach to medical education.

Francis R. Fraser

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Notes on Contributors

DR J. F. ACKROYD was formerly Lecturer in Pathology, University of Bristol, and Lecturer in Pathology, St Mary's Hospital Medical School, London. He is now Senior Lecturer in Medicine at St Mary's Hospital Medical School. Dr Ackroyd's work has been mainly concerned with the analysis of the mechanisms of different kinds of purpura, and among his publications are: "The pathogenesis of thrombocytopenic purpura due to hypersensitivity to sedormid" (*Clin. Sci.* 1949, **7**, 249), "Allergic purpura, including purpura due to foods, drugs and infections" (*Amer. J. Med.* 1953, **14**, 605), and "Thrombocytopenic purpura due to hypersensitivity to the antihistaminic drug antazoline (2-(*N*-phenyl-*N*-benzylaminomethyl)-imidazoline)" (*Sang*, 1955, in press).

DR KENNETH BAILEY is an authority on proteins and is particularly interested in the proteins of muscle and in those concerned with blood clotting. He is co-editor of *Advances in protein chemistry* and also of a comprehensive treatise, *The proteins: chemistry, biological activity, and methods* (1953). He is an assistant director of research in biochemistry at the University of Cambridge and a lecturer and Fellow of Trinity College. Dr Bailey has previously contributed to the Bulletin a paper entitled "Fibrous proteins as components of biological systems" (*Brit. med. Bull.* 1948, **5**, 338), with which a biographical note was published.

DR F. R. BETTELHEIM worked in the Department of Biochemistry, Cambridge, from 1950 to 1953 with Dr K. Bailey on the mechanism of the clotting of fibrinogen. During the year 1953–54 he held the position of Research Associate in the Department of Biochemistry, University of Washington, Seattle, USA, his work being concerned chiefly with the mechanism of the activation of chymotrypsinogen. He is now back in Cambridge as a Fellow of King's College, engaged on problems of protein chemistry. The following are three of his publications: "Tyrosine-O-sulfate in a peptide from fibrinogen" (*J. Amer. chem. Soc.* 1954, **76**, 2838); with K. Bailey, "The products of the action of thrombin on fibrinogen" (*Biochim. biophys. Acta*, 1952, **9**, 578); with H. Neurath, "The rapid activation of chymotrypsinogen" (*J. biol. Chem.* in press).

DR ROSEMARY BIGGS has worked as Assistant Pathologist at the Radcliffe Infirmary, Oxford, for 10 years. During this time she has been interested in the reliability of haematological methods and has published papers on this subject in collaboration with Dr R. L. MacMillan (*J. clin. Path.* 1948, **1**, 269; 288). She has also worked on problems of blood coagulation, including the laboratory control of anticoagulant therapy and the nature of the clotting defect in haemophilia and related conditions, and has published, jointly with Dr R. G. Macfarlane, *Human blood coagulation and its disorders* (1953).

DR C. C. BURT was, from 1924 to 1938, Lecturer in Biology at the Lady Hardinge Medical College for Women, New Delhi, India. During the Second World War she was on the staff of a peripheral nerve and peripheral vascular injuries unit in an Emergency Medical Service hospital under the charge of Sir James Learmonth. Later Dr Burt was a research assistant in the Medical Research Council's Clinical Endocrinology Research Unit, University of Edinburgh. At present she is Assistant to Sir James Learmonth in his peripheral vascular clinic at the Royal Infirmary, Edinburgh. Her published papers include "Peripheral skin temperature in normal pregnancy" (*Lancet*, 1949, **2**, 787); "Arteriography in lesions of peripheral vessels" (*J. Fac. Radiol., Lond.* 1952, **3**, 223); "Anticoagulants" (*Edinb. med. J.* 1954, **61**, 273).

DR J. V. DACIE has, since 1946, been on the staff of the Postgraduate Medical School of London, first as Senior Lecturer and now as Reader in Haematology. In 1937 he held a Medical Research Council Postgraduate Studentship, and from 1938 to 1939 was Will Edmonds Clinical Research Fellow. From 1939 to 1942 he was a pathologist in the Emergency Medical Service. He was in the Royal Army Medical Corps from 1943 to 1946, and held the ranks of major and then lieutenant-colonel. He is author of *Practical haematology* (1950) and *The haemolytic anaemias: congenital and acquired* (1954) and is editor of the *British Journal of Haematology*, which starts publication at the beginning of 1955.

DR A. S. DOUGLAS has been Lecturer in Medicine in the University Department of Medicine, Royal Infirmary, Glasgow, since 1953. From 1945 to 1948 he served with the Royal Army Medical Corps. From 1951 to 1953 he held a Medical Research Council fellowship in clinical research and worked with Dr R. G. Macfarlane and Dr Rosemary Biggs at the Radcliffe Infirmary, Oxford. His research has been, and still is, largely concerned with blood coagulation and allied problems. He is joint author of "Effect of vitamin-K preparations on hypoprothrombinaemia induced by dicoumarol and Tromexan" (*Brit. med. J.* 1952, **1**, 412), "Christmas disease: a condition previously mistaken for haemophilia" (*Brit. med. J.* 1952, **2**, 1378) and "The thromboplastin generation test" (*J. clin. Path.* 1953, **6**, 23).

PROFESSOR J. B. DUGUID has held teaching appointments in pathology since 1922, firstly in Aberdeen and subsequently in Manchester, Cardiff and Newcastle, having been appointed to the Chair of Pathology in the Welsh National School of Medicine in 1932, and to that in the University of Durham in 1948. He began the study of arterial diseases under the late Professor T. Shennon in Aberdeen, and in 1925 was awarded highest honours in the M.D. degree for a thesis on atheroma of the aorta. Professor Duguid's interests have ranged over a wide variety of subjects from the classification of chronic nephritis to a tapeworm infection in trout, but his main interest has been arterial disease, and his chief contribution to pathology has been his revival of the thrombosis hypothesis of Rokitansky, outlined in his article in this number of the Bulletin.

DR C. HOUGIE is, at present, Senior Registrar in Pathology at the West London Hospital. From June 1952 to February 1954 he was a Dan Mason Research Fellow at the West London Hospital Medical School, where he was engaged primarily in the study of problems relating to blood coagulation, with special reference to the role of platelets. He has published articles entitled "Tromexan therapy, dosage and indications" (*Lancet*, 1951, **2**, 1118), "Pseudo-haemophilia: an acquired haemorrhagic diathesis due to a circulating anticoagulant" (*J. clin. Path.* 1953, **6**, 30) and, jointly with Dr M. E. Fearnley, "Nature and action of circulating anticoagulants" (*Acta haemat.* 1954, **12**, 1).

PROFESSOR R. B. HUNTER has been Professor of Pharmacology and Therapeutics in the University of St Andrews, Dundee, since 1948. He is also Physician to Maryfield Hospital, Dundee, Adviser in Therapeutics to the Eastern Regional Hospital Board (Scotland), a member of the Secretary of State for Scotland's Advisory Committee on Medical Research, and Convener of the Postgraduate Medical Studies Committee of the University of St Andrews. He is at present engaged on research concerned with blood coagulation, the action of anticoagulant drugs and their use in the treatment of thrombo-embolic diseases, the action of drugs on the pulmonary circulation in man, and the study of endocrine diseases, principally those affecting the pituitary and the adrenal glands. Professor Hunter was previously Lecturer in Therapeutics at the University of Edinburgh, and Assistant Director of the Edinburgh Post-Graduate Board for Medicine. In 1948 he was awarded a Commonwealth Fellowship in Medicine, but this he resigned. In 1950 he was Visiting Lecturer in Physiology and Pharmacology in Boston University School of Medicine. Professor Hunter presented a paper entitled "Observations on Factor X", at the First International Conference on Thrombosis and Embolism, which was held in Basle in 1954. He has jointly published the following papers: "A review of antihistamine drugs" (*Quart. J. Med.* 1948, n.s. **17**, 271), and "The function of the organs of Zuckerkandl" (*Clin. Sci.* 1953, **12**, 317); he has also contributed two chapters to the forthcoming edition of Price's *Textbook of medicine* (9th ed., in press).

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NOTES ON CONTRIBUTORS

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DR D. M. SHEPHERD is Lecturer in Pharmacology in Queen's College, University of St Andrews, Dundee. He was formerly a member of the research department of Imperial Chemical Industries Ltd. (Nobel Division), and subsequently received the Ph.D. degree of the University of St Andrews, for research in organic chemistry. In 1950 he turned his attention to the application of chemical techniques, especially paper chromatography, to pharmacological problems. In this field his publications, mainly in collaboration with other authors, include studies of the distribution and origin of adrenaline in the organs of man and various animals. More recently he has directed his attention to the chemical mechanisms underlying drug actions, with particular reference to the mode of action of coumarin anticoagulants and of organic nitrates.

DR KENNETH WALTON qualified at University College Hospital, London, where he held a demonstratorship in pathology. After house appointments at Addenbrooke's Hospital, Cambridge, he served in the Royal Army Medical Corps as a pathologist in hospitals and field laboratories in India and Hong Kong. On leaving the Army with the rank of major, he returned for a year to the Morbid Anatomy Department of University College Hospital. For the last six years he has worked in the Department of Experimental Pathology at the University of Birmingham, where he now holds the appointment of Senior Lecturer. His published work on general pathological topics includes papers on cerebral tumours, congenital cardiac valvular abnormality, eosinophilic arteritis, and psittacosis. He is currently interested in the biological behaviour and pathological effects of natural and synthetic charged macromolecules. His publications in this field include a review on dextran and its derivatives, a series of papers on the development of the synthetic heparinoid, dextran sulphate, and an account of the investigation of the histochemical basis of metachromasia. Dr Walton is a member of a Medical Research Council subcommittee appointed to advise on the establishment of biological standards for materials used in the treatment of blood coagulation disorders. He has recently been awarded a Rockefeller travelling fellowship in medicine and is working in the Department of Physical Chemistry Related to Medicine at Harvard University, on plasma lipoproteins in relation to blood coagulation and atherosclerosis.

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Scientific Editor: *A. S. Parkes*

Fifty Years of Reproductive Endocrinology

Fifty years ago today, on 18 May 1905, F. H. A. Marshall, in conjunction with W. A. Jolly, read before the Royal Society of London a paper entitled " Contributions to the physiology of mammalian reproduction. Part 2. The ovary as an organ of internal secretion." [1] This paper included the following sentences:

> The Mammalian ovary, in addition to its function of producing ripe ova, is an organ elaborating an internal secretion which reacts on the general metabolism of the animal, as is shown by the effects brought about by its removal. The secretion is probably formed in greater or less quantity at all times, but it is produced in greatest abundance at certain more or less regularly recurrent periods, when it brings about those conditions which characterise the pro-oestrum and oestrus. It is at these periods also in typical cases that the ova mature and the follicles discharge.
>
> . . . After ovulation, which takes place during oestrus, the corpus luteum is formed, and this organ provides a further secretion whose function is essential for the changes taking place during the attachment and development of the embryo in the first stages of pregnancy.

[1] Marshall, F. H. A. & Jolly, W. A. (1906) *Phil. Trans.* B, **198**, 99.—Ed.

At the time these far-sighted words were written no demonstrably active extracts of the gonads had been prepared, and the experimental demonstration that the gonads were organs of internal as well as of external secretion was far from complete. The most noteworthy thing about this quotation, however, is that it embodies the first clear recognition of the respective roles of the follicular and luteal hormones, a recognition which avoided alike the current tendency to over-emphasize the function of the corpus luteum and the later tendency to regard the oestrus-producing substance as the main or only ovarian hormone. Against the background of contemporary knowledge the words written by Marshall are an outstanding example of scientific prescience, and, on the occasion of the publication 50 years later of a number of the *British Medical Bulletin* devoted to " Hormones in Reproduction ", it is fitting to recall that the study of reproductive endocrinology in Great Britain was guided so wisely at such an early stage.

In the 15 years following the publication of Marshall's paper little progress was made with the preparation of active extracts of the gonads, though further evidence was obtained of their endocrine function. The years between the two wars, however, especially the middle decade, saw astonishing advances. In this period, potent preparations of the oestrus-producing hormone were developed by Edgar Allen and Doisy, and crystalline oestrogens isolated independently by Doisy, by Butenandt, and by Marrian; androgenic extracts of testis were prepared by Moore and Koch, and crystalline testosterone was isolated in Laqueur's laboratory. During the same period, the classic work of Fraenkel and of Bouin was brought to fruition by the preparation of active extracts of the corpus luteum by Corner and Willard Allen, and by the isolation of progesterone. The chemical constitution and relationship of the three gonadal hormones and of many variants of oestradiol and testosterone were worked out with great speed, because of the happy coincidence, to which R. K. Callow (p. 126) draws attention, that decisive advances had just been made in related fields of steroid chemistry.

Simultaneously, exciting things had been happening in another direction. Observations on the rapid maturation of immature ovaries grafted to adult animals, and on compensatory hypertrophy of one ovary after removal of the other, had long fostered the idea that the gonads were subject to some extragonadal control. Experiments on the effects of removing the pituitary gland had also been suggestive, as had Evans' work with extracts of the anterior lobe. It remained, however, for Smith and Engle, and almost simultaneously Zondek and Aschheim, to bring these two lines of work together and to show that the anterior pituitary body contained substances which would cause follicular maturation, ovulation and corpus luteum formation in the ovary, and that the anterior pituitary body was probably, therefore, the immediate seat of gonadal control.

Rarely has a single subject of biological research blossomed so profusely in so short a time. The fruit is now before us as an impressive body of knowledge festooned, as might be expected, with loose ends of observation and deduction.

The situation in the male may be regarded as comparatively straightforward, though there are still unsolved problems such as the role, if any, of the testicular oestrogens in the sexual endocrinology of the male, and the question of whether liaison with the anterior pituitary body involves a third internal secretion by the testis. In the female the endocrine integration of the reproductive processes is naturally far more complicated than in the male. Among eutherian mammals and, as E. C. Amoroso reminds us, among many lower vertebrates, the production of living young involves a series of accessory organs controlled by two ovarian hormones which are essentially cyclic in action and secreted under the influence of two or more hypophyseal gonadotrophins, the supply of the latter being regulated through the hypothalamus partly by external factors, partly by events in the accessory reproductive organs and partly by unknown agencies. Moreover, during pregnancy, ovarian and gonadotrophic hormones are reinforced by substances of placental or endometrial origin. To all this must be added the facts that our knowledge of the action of the sex hormones at cellular level is rudimentary, that differences in reproductive endocrinology between different species are very great, and that under certain conditions or at certain stages other endocrine organs, such as the adrenal cortex and the posterior pituitary lobe, become involved.

The planning of a number of the Bulletin to cover such a vast field presented certain difficulties. It was intended originally to make a comprehensive schedule of the whole subject, and to allocate the various sections to appropriate authors. This proved too large a project, and in the event each author was asked to contribute an article on a subject known to be among his special interests. This arrangement has produced papers of a high level of authority, though it has led to some oddities of subdivision and some slight overlapping and omission.

It remains only to thank the contributors for their co-operation and to hope that " Hormones in Reproduction " will provide a useful exposition of current knowledge in this intriguing field of research. *A. S. Parkes*

This number of the *British Medical Bulletin* was planned at a meeting of a small Committee nominated by Professor G. W. Harris, and consisting of Dr S. J. Folley, Dr John Hammond, sr, Dr A. S. Parkes and Dr G. I. M. Swyer. In the unavoidable absence of Professor Harris, Dr Parkes took the Chair and agreed to act as Scientific Editor.

We are much indebted to Dr Parkes for his help and for his advice on the many technical problems that arose in the course of preparing this symposium.

Notes on Contributors

DR E. C. AMOROSO has been Professor of Physiology at the Royal Veterinary College, in the University of London, since 1948. He graduated in medicine at University College, Dublin, and studied at the Kaiser Wilhelm Institut für Zellforschung, Berlin, at the University of Freiburg, and at University College, London. In 1929 he was awarded the McArdle Medal in Surgery and a travelling fellowship in science at the National University of Ireland. Six years later he was appointed to the staff of the Royal Veterinary College. In 1954 he visited the USA at the invitation of the Josiah Macy, Jr Foundation and the Carnegie Institution of Washington. He was appointed visiting Professor of Anatomy at Washington University, St Louis, and delivered the 1953–1954 Goldwin Smith Lectures at Cornell University, Ithaca. Professor Amoroso's work since 1935 has covered aspects of the foetal circulation and the early development of the mammalian egg, but during the last ten years his main interests have been in the structure and function of the placenta and in the physiology of reproduction. He is the author of the chapter on the placenta in the 3rd edition of *Marshall's physiology of reproduction* (1952), and of papers in the *Proceedings of the Royal Society* and in various other scientific journals.

DR R. K. CALLOW, sometime Exhibitioner of Christ Church, Oxford, held an industrial post for a short time and then returned to Christ Church as a Senior Scholar. He has been in the service of the Medical Research Council at the National Institute for Medical Research, London, since 1929. During the Second World War, from 1940 to 1945, he joined the Royal Air Force and was mentioned in despatches for service on the north-west frontier of India. He served a full term of seven years on the Editorial Board of the *Biochemical Journal*, finally as Deputy Chairman. His published work, jointly with other authors, includes papers on physical organic chemistry, alkaloids, calciferol, the rickets-producing action of cereals, sex hormones, optical activity of the steroids, clinical endocrinology, antibiotics, and the chemistry of adrenocortical hormones, and he is engaged on work on the steroidal sapogenins, arising from the discovery that sisal is a source of hecogenin for the commercial manufacture of steroids.

DR B. A. CROSS, on graduation from the Royal Veterinary College, London, came to St John's College, Cambridge, with a research scholarship of the Animal Health Trust. After completing the B.A. (Honours) in physiology he commenced research in the Physiological Laboratory, Cambridge, under Dr G. W. Harris, on the role of the posterior pituitary gland in lactation. This work, which was carried out during the successive tenure of an Imperial Chemical Industries and a Wellcome Trust research fellowship, led to the Ph.D. degree and the award of the Gedge Prize in Physiology. In 1955 he was appointed University Lecturer in Veterinary Anatomy in the Department of Zoology, Cambridge. Present research work is concerned with the physiology of oxytocin in reproductive as well as in lactational processes, and is supported by a research grant from the Agricultural Research Council. He is a member of the Physiological Society and the Society for Endocrinology, and is Assistant Editor of the *British Journal of Animal Behaviour*.

SIR CHARLES DODDS was educated at the Middlesex Hospital, London, where he was appointed Head of the Biochemical Department in 1922. In 1925 he was appointed Courtauld Professor of Biochemistry in the University of London, and in 1928 Director of the Courtauld Institute of Biochemistry, then newly built. He is the Harveian Librarian of the Royal College of Physicians, and a Sir Arthur Sims Travelling Professor of the Royal College of Surgeons. His scientific work has consisted in a study of subjects connected with chemical pathology, and particularly of synthetic oestrogenic substances. His publication on this subject, written in conjunction with J. W. Cook & C. L. Hewett, appeared in 1933 (*Nature, Lond.* 1933, **131**, 56). A series of investigations in collaboration with W. Lawson indicated that relatively simple substances possessed powerful oestrogenic activity, and these investigations pointed the way to the possible high activity of the symmetrical dimeride of anol. This was eventually synthesized in collaboration with Sir Robert Robinson and his colleagues in 1938, and is the substance known as stilboestrol today. Sir Charles has contributed two previous papers to the Bulletin: the first, on morphine substitutes, appeared in *British Medical Bulletin*, 1946, **4**, 88; the second, a note on the carcinogenic action of stilbene derivatives, in *British Medical Bulletin*, 1947, **4**, 378.

DR BERNARD DONOVAN is a physiologist at the Institute of Psychiatry, Maudsley Hospital, London, engaged on research into hypothalamic-pituitary relationships. After graduating from University College, London, he held a Medical Research Council studentship and was awarded a Beit Memorial Fellowship in 1954. His investigations include studies on the control of follicle-stimulating hormone secretion in the ferret, and of luteinizing and lactogenic hormones in the rabbit.

PROFESSOR C. W. EMMENS was on the staff of the Medical Research Council at the National Institute for Medical Research, London, from 1937 to 1948, and was seconded during the Second World War to the Home Office and to the Royal Air Force for research on air warfare. He went to the University of Sydney, Australia, in 1948 to form a new Department of Veterinary Physiology, and was elected to the Chair in 1950. In 1952 and 1953 he also directed the Commonwealth Scientific and Industrial Research Organization's Sheep Biology Laboratory at Prospect, New South Wales. His research has been on sex hormones and other aspects of endocrinology, and on spermatozoa, with particular interest in bioassay. He is at present working on the mode of action of oestrogens and the deep freezing of spermatozoa. Professor Emmens was Regional President of the Biometric Society in 1951 and 1952, President of the Sydney Association of University Teachers in 1954, and an Australian delegate to the XIXth International Physiological Congress, which was held in Montreal in 1953. His publications include *Principles of biological assay* (1948), review articles, and some 70 scientific papers; he is editor of *Hormone assay* (1950).

DR S. J. FOLLEY has previously contributed to the Bulletin (*Brit. med. Bull.* 1947, **5**, 130, 135 and 142) three articles on the physiology of the mammary gland, with which a biographical note was published. He is at present Head of the Physiology Department of the National Institute for Research in Dairying, Shinfield, Reading, with the rank of Deputy Chief Scientific Officer, and in 1953 he was a Visiting Professor at the Collège de France. He was elected a Fellow of the Royal Society in 1951.

DR JOHN HAMMOND, SF, was Honorary Director of the Agricultural Research Council Unit of Animal Reproduction from 1946 to 1954. Since his retirement from that Unit he has been employed jointly by the Ministry of Agriculture and the Agricultural Research Council. From 1920 to 1943 he was Physiologist at the Animal Nutrition Institute, School of Agriculture, Cambridge, and from 1943 to 1954 he was Reader in Agricultural Physiology at the University of Cambridge. Having been a Fellow of Downing College, Cambridge, from 1936 to 1954, he was made an Honorary Fellow in 1954. He became a Fellow of the Royal Society in 1933. Dr Hammond has served on a number of committees concerned with livestock in all parts of the world. He was President of the Nutrition Society from 1947 to 1950, and of the Agricultural Section of the British Association for the Advancement of Science, at Liverpool, in 1953. He is a member of the editorial board of several agricultural journals and author of many scientific papers, and among his best-known books are the following: *The physiology of reproduction in the cow* (1927), *Growth and development of mutton qualities in the sheep* (1932), and *Farm animals: their breeding, growth and inheritance* (2nd ed., 1952). He is also editor of *Progress in the physiology of farm animals* (1954).

PROFESSOR G. W. HARRIS has been University Lecturer in Anatomy, and in Physiology, at the University of Cambridge, and now holds the Fitzmary Chair of Physiology at the Institute of Psychiatry, Maudsley Hospital, London. In 1950 he was appointed Claude Bernard Visiting Professor of Anatomy at the University of Montreal, and he has recently returned from Berkeley where he was Visiting Professor in Anatomy at the University of California. In 1953 he was elected a Fellow of the Royal Society. Professor Harris has been particularly concerned with investigations into the physiology of the hypophyseal portal system, and has published numerous papers in this field. He has reviewed progress in this subject in *Physiological Reviews* (*Physiol. Rev.* 1948, **28**, 139), and has recently completed a study entitled "Neural control of the pituitary gland", which is in course of publication. He is at present leading investigations into the neural control of the secretion of the trophic hormones of the pituitary gland. An earlier paper by Professor Harris, entitled "The hypothalamus and endocrine glands", appeared in *British Medical Bulletin*, 1950, **6**, 345.

DR CHESTER JONES is a Lecturer in Zoology at the University of Liverpool. In 1947 he was awarded a Commonwealth Fund Fellowship and, during the two-year tenure at Harvard, he published his findings on the influence of pituitary gonadotrophins on the adrenal cortex. More recently he has clarified the problem of the mouse X zone during first pregnancy (see *Proc. roy. Soc.* B, 1952, **139**, 398), and published a series of papers, jointly with other authors, on the correlation of structure and function in the adrenal cortex (*J. Endocrin.* 1954, **10**, 245, 251, 262, 266). Over the past three years he has been examining adrenocortical-neurohypophyseal relationships in the control of water and salt-electrolyte metabolism. In addition, Dr Chester Jones supervises a research group investigating problems in comparative endocrinology and he organized the first international symposium on this subject, which was held at the University of Liverpool in July 1954.

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Second World War, after working in Anti-Aircraft Command and in the Telecommunications Research Establishment, he undertook radar liaison duties with the Royal Air Force. In 1946 Dr Harrison Matthews became a Research Fellow of the University of Bristol. He is the author of several books and numerous scientific papers on zoological subjects in journals of learned societies.

PROFESSOR C. J. O. R. MORRIS was appointed to the Chair of Experimental Biochemistry at the London Hospital Medical College in 1954. He was previously Reader in Chemical Pathology at that College. He was educated at University College, Cardiff, and worked at the Lister Institute of Preventive Medicine, London, during 1932–36, and at the Kaiser Wilhelm Institut für medizinische Forschung, Heidelberg, under Professor Richard Kuhn, during 1936–37. During the latter period he worked on the synthesis of polyenes, including vitamin A. His more recent researches have been concerned with the chemistry and determination of protein, polypeptide and steroid hormones, with special reference to the pituitary adrenocorticotrophic hormone (ACTH) and the adrenocortical steroids. Professor Morris has been particularly interested in the application of physico-chemical methods in biochemistry. Recent work includes studies on the chemistry of adrenocorticotrophic hormone in *Recent Progress in Hormone Research* (vol. 7, 1952), and a series of papers on the polarographic estimation of various steroid hormones in the *Biochemical Journal*.

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DR A. S. PARKES is a member of the Scientific Staff of the Medical Research Council at the National Institute for Medical Research, London. He is a Fellow of University College, London, and in 1933 was elected a Fellow of the Royal Society. From 1923 to 1924 Dr Parkes was Sharpey Scholar, University College, London; from 1924 to 1927 he was a Junior Beit Memorial Research Fellow, and from 1928 to 1930 a Senior Fellow. In 1926 he was awarded the Schäfer Prize in Physiology. He held a Julius Mickle Fellowship in the University of London, 1929, was a Foulerton Student of the Royal Society from 1930 to 1934, Sidney Ringer Lecturer, University College Hospital, London, 1934, and Ingleby Lecturer, University of Birmingham, 1940. From 1949 to 1950 Dr Parkes was President of the Section of Endocrinology, Royal Society of Medicine; he has been Chairman of the Society for Endocrinology (1944–51) and of the Society for the Study of Fertility (1950–52). He has contributed papers to scientific journals, on the physiology of reproduction, on endocrinology, and on the behaviour of living cells at low temperatures; and is author of *The internal secretions of the ovary* (1929), and editor of *Marshall's physiology of reproduction* (3rd ed., 1952).

DR E. R. PLUNKETT has been Lecturer in the Department of Medical Research of the University of Western Ontario, London, Canada, since 1949, and is also at present engaged in part-time clinical endocrine research at the Ontario Cancer Treatment and Research Foundation Clinic, Victoria Hospital, London, Ontario. He is a Fellow of the Department of Medicine and of the Department of Obstetrics and Gynaecology of the University of Western Ontario. From 1951 to 1952, he was Resident and Research Fellow, Division of Endocrinology, Duke University, North Carolina, USA. Dr Plunkett was awarded the Cairncross and Lawrence Prize for his Ph.D. thesis in 1950, and the R. S. McLaughlin Fellowship in 1951. Some of his publications, with other authors, are: "Factors affecting the control of the pituitary gland" (*Recent Progr. Hormone Res.* 1950, **5**, 263); "The effects of injection of *Lithospermum ruderale* on the endocrine organs of the rat" (*Endocrinology*, 1951, **49**, 1); "Direct hormone inactivation by extracts of *Lithospermum ruderale*" (*J. Endocrin.* 1954, **10**, 212).

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THE EFFECT OF EXTERNAL STIMULI ON THE BREEDING-CYCLE OF BIRDS AND MAMMALS

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A number of facts about animals suggest the intervention of some agencies tending to adjust their reproductive activities to a special season of the year, thus ensuring that the young are born at the most propitious time (F. H. A. Marshall, 1936, 1942). Analysis of breeding records in many species has established that sexual periodicity is influenced by many extrinsic and intrinsic factors; and of the environmental factors that influence reproduction, and "anchor" the breeding-cycle, light and temperature are the most important. There does seem, however, to be evidence that environmental experiences derived from association of the sexes may also stimulate the seasonal cycle in photoperiodically regulated animals. Ovulation in many birds and mammals follows upon a more or less complicated train of courtship behaviour and probably would not occur in the absence of such sexual stimuli.

Within the body, on the other hand, it has been shown that the changes in the reproductive organs that regulate the psychological and physiological aspects of the breeding behaviour of mammals and birds are conditioned by the internal secretions of the gonads; and that the activity of the gonads is in turn predominantly under the control of the adenohypophysis. It would thus appear that the pituitary gland is a mediating structure through which the organism adjusts its reproductive life to environmental change (Zuckerman, 1947).

1. Light

Encouraging egg production by subjecting hens to artificial lighting by night, now an established practice in poultry husbandry, seems to have been employed by the Spaniards for at least a century (Rowan, 1936). But it was not until Rowan's studies (Rowan, 1925, 1926, 1929, 1930, 1931, 1932, 1936, 1938) on migration in the junco, in some finches and in the American crow, and those of Bissonnette (1930, 1931a, 1931b, 1932, 1933) on the European starling, showed that the coincidence of gonadal recrudescence with increasing hours of daylight and northward migration was no accident, that photoexperimentation became a subject of major scientific interest.

Rowan's experiments on the junco demonstrate that, while food supply, weather conditions and temperature may be contributory factors in determining the sexual and migratory activities of this bird, it is the recurring periods of light and darkness that act as the proximate environmental stimulus; both sexes are equally dependent on variation in exposure to light for the seasonal changes of activity in the sex gland. Rowan (1938) believed, moreover, that extra illumination induced gonadal differentiation during periods of sexual quiescence by permitting the birds to indulge in increased exercise. This view, subsequently modified by substituting "wakefulness" for physical activity, retains certain adherents (Wolfson, 1949), but subsequent studies by others did not give such importance to increased exercise, and Burger (1949) was able to write that "no experiment thus far devised has proven that any external stimulus, *operating in darkness*, induces precocious gametogenesis."

a. Quality and Quantity of Light

Other workers have confirmed and extended Rowan's observations on the stimulating action on the testis of increasing periods of light. Thus, Bissonnette (1930, 1931a, 1931b) studying the European starling, and Benoit (Benoit, 1936, 1937; Benoit & Ott, 1944) the domestic duck, found that light of long wavelength was most effective for supplanting natural daylight and that green light exerted a definite inhibitory effect upon the gonads. In addition, they found that intensity of light was an important factor, but that although increased lighting induced sexual activity it would not maintain it indefinitely. An important aspect of Benoit's experiments is that he showed that there is a quantitative relation between the gonadal response of drakes and the duration and intensity of illumination, but that neither hypophysectomy nor shielding the eyes of normal birds prevented a response. Wolfson (1953) found likewise that in the white-throated sparrow there was increased reproductive response in the ovary and oviduct when the birds were subjected to a six-hour cycle of light and darkness. This response was greater than in birds exposed continuously to 20 hours of illumination a day.

b. The Relationship between Latitude and Breeding-Season in Birds

The duration and intensity of light, though important, are not the only factors involved in the seasonal activities in birds. We have no precise information on the responses of equatorial birds to light increments; it would seem reasonable, however, to suppose that on the equator, where the days and nights are approximately of equal length throughout the year, the fluctuations are too small to be an effective factor. Moreover, the observation that certain transequatorial migrants move northward from regions where the days are increasing in length is sufficient to suggest that factors other than light increments are involved in tropical and equatorial species. It may, therefore, be necessary to postulate some degree of inherent rhythm unaffected by external environmental factors,

in order to account for the reproductive behaviour of such tropical birds as the sooty tern, investigated by Murphy (1936). With this species there is evidence that the nesting season on Ascension Island begins a little earlier in each successive year and that the change in time is sufficient to make the birds breed on the average four times within three years. On the other hand, although there are facts pointing to an inherent seasonal cycle in photoperiodically regulated animals, there seems to be evidence of the cycle being reset by factors other than light, since Fraps, Neher & Rothchild (1947) showed that under constant light the rhythm of egg-laying in the domestic fowl could be reset by changing the times of feeding.

Murphy (1936) also records some facts about the periodic breeding of certain penguins, which are in striking contrast to those recorded for the great majority of birds. Thus the Peruvian and African penguins that have invaded warm-temperate and tropical environments may be occupied in breeding activities throughout the whole year and would appear to be unusual among birds in having freed themselves from external seasonal influences. Again, as Betts (1952) has shown, the majority of the species of birds in South India have a breeding season which is little more extended than that of birds in temperate climates, although the country is well within the tropics and the climate is presumably favourable to breeding throughout the greater part of the year. Seasonal breeding in tropical birds has also been studied by Bannerman (1930, 1931, 1933), who suggests that it is determined by the recurrence of rains and other ecological factors which may also act, through the nervous system, upon the anterior pituitary.

The relationship between latitude and breeding season in birds has been studied by Baker (1938). He has shown that ". . . as one goes north from temperate latitudes one finds a general tendency for egg-laying to start later and later at the rate of some 20 or 30 days per 10° latitude." Conversely, as one goes south from the temperate latitudes towards the tropical and equatorial zones, there is a tendency for many species of birds to start egg-laying earlier and earlier.

c. Photo-Stimulation and Sexual Periodicity in Mammals

As with birds, some seasonally breeding mammals are known to show the same sensitivity to waning and waxing of daylight hours, whereas others, for example the guinea-pig (Dempsey, Meyers, Young & Jennison, 1934), and the ground-squirrel (Moore, Simmons, Wells, Zalesky & Nelson, 1934), do not. Sexually inactive ferrets, as Bissonnette (1932), Hill & Parkes (1933), F. H. A. Marshall (1940), Jefferson (1940), Thompson (1951, 1954), Thompson & Zuckerman (1953, 1954, 1955) and Donovan & Harris (1954) have shown, become reproductively active in winter when subjected to artificially lengthened periods of light, females being much more completely controlled in this way than males. In rats also (Fiske, 1941), it appears that light causes a greater output of follicle-stimulating hormone than of luteinizing hormones, since females receiving additional rations of light remain in an oestrous condition for several weeks instead of the normal period of about 14 hours. Baker & Ranson (1932a, 1932b, 1933) showed that the oestrous cycle in the vole could be modified by varying the ration of light, though food and probably latitude might alter the response (winter type of food being best; earlier activity farther north). In the hedgehog (Allanson & Deanesly, 1934), sexual activation was induced by extra illumination, and also by increases in temperature and other factors. Again, racoons can be induced to breed in December instead of February by increasing autumn lighting (Bissonnette & Csech, 1937), and the same would appear to be true of the white-footed mouse (Whitaker, 1936). In general, however, a species indigenous to one hemisphere adjusts its breeding-season to the new calendar when it is moved across the equator, so that within a year or two the time of the new breeding-season coincides with the previous period of sexual repose (Roux, personal communications to F. H. A. Marshall, 1936; F. H. A. Marshall, 1937).

Hart (1951) investigated the effects of a fixed dark-light rhythm without the necessity of a gradual reduction in the amount of light, and found that, in the ferret, oestrus and anoestrus can be induced by influencing the activity of the pituitary through a contrast-sensitive mechanism which is stimulated by the ratio of light to dark periods. Two parts of light to one of darkness induces oestrus; the reverse, anoestrus. Slowly altering amounts of light, and the total daily quantity, are not controlling factors, for increasing the light-dark stimuli from one to two a day produces no acceleration in the onset of oestrus. Anoestrus can be induced more quickly than oestrus.

The seasonal changes of light environment have a number of different results. Bissonnette (1941) reported that, in certain breeds of goats, increasing daily light periods from 25 January to 5 April, followed by decreasing light until 5 July, resulted in the cessation of oestrous cycles in February instead of March and the resumption of oestrus in May and June instead of September. It is evident, therefore, that in the same animal light can have double effects: promoting gonadal activity, or bringing the breeding-season to a close and blocking further stimulation. Moreover, many wild mammals, such as the field mouse, racoon and ground-squirrel, have "long-day" breeding seasons in the spring, whereas others, like the sheep, goat and deer, breed during the autumn or "short-day" season. If light is regarded as stimulatory to the former, then it is inhibitory to the latter.

Finally, the case of the nocturnal animal in the wild state, which is compelled to spend daylight hours in the darkness of a burrow, should not be overlooked. The polecat, the wild ancestor of the domestic ferret, for example, usually emerges only at twilight and consequently its ration of light must be considerably reduced, yet there is no reason to suppose that the broad pattern of the photoperiodic phenomena differs in the wild and domestic races.

d. The Refractory Period

We do not know with certainty what specific factors, either external or internal, operate in bringing about the refractory period that follows breeding in many birds and mammals, and in which it is impossible, however favourable the environment, for another sexual cycle to be started. The refractory period in birds was investigated by Riley (1936, 1937), who found that treatment with light stimulated both adult and immature male sparrows in November; if, however, treatment was started seven weeks earlier, adults failed to respond, though males hatched in the same season could do so. Refractoriness has also been detected in the starling (Burger, 1947), duck (Benoit, Assenmacher & Walter, 1950), golden-crowned sparrow (Miller, 1951), and in the junco (Wolfson, 1952). The existence of refractoriness in the mink is also claimed by Hammond (1951, 1954). The view of refractoriness proposed by A. J. Marshall (1950, 1951, 1954) is based

upon histological changes in the avian testis under natural lighting. This author believes that refractoriness is due to the end of the life-cycle of one generation of Leydig cells, and that its duration is determined by the time required for the next generation to mature. It is, however, possible that the gonads merely reflect pituitary activity.

e. The Mode of Action of Light

Many workers have given their attention to elucidating the problem of the means by which light stimuli are transmitted to the pituitary, but no definite conclusion has yet been reached. Benoit (1936, 1937) and Benoit & Ott (1944) found that light directed upon the head of the drake is effective in evoking the pituitary response when all other parts of the body are shielded. They further found that light may stimulate the anterior hypophysis through the eyes by excitation of deeply seated nerve centres, or by acting directly on the gland after penetrating the overlying tissues. Neither section of the optic nerves nor removal of the eyes decreases the response to intense light. Narrow beams of light acting directly upon certain parts of the hypothalamus stimulate the pituitary gland and activate the testis; activation of the gonads may also be induced by focusing light directly upon the anterior hypophysis through a narrow tube or a quartz rod placed in the empty orbit.

Bissonnette's experiments with the ferret (Bissonnette, 1930, 1931a, 1931b, 1932, 1933) indicate that the eyes are the avenues of reception; unlike the duck, the ferret does not respond to light after the optic nerves have been cut, the animal still displaying an inherent sexual cycle which is independent of light and hence does not progress in accordance with seasonal changes. F. H. A. Marshall (1940) suggests that stimulation by ultra-violet irradiation may result not only in the release of the follicle-stimulating hormone, but also of the luteinizing fraction of the pituitary secretion, the former, however, always being dominant. Hill & Parkes (1933) demonstrated that, after hypophysectomy, ferrets do not come on heat even though they are artificially irradiated, the stimulus probably being transferred from the retina to the pituitary.

Since the integrity of the optic nerves appears to be essential to the response of the ferret's gonad to light, the problem has been to discover whether nerve fibres from the optic pathway enter the anterior lobe of the pituitary. The experiments of Clark, McKeown & Zuckerman (1939) failed to provide the answer to this specific question, but led to the conclusion that, although the integrity of the optic nerve is necessary, neither the visual cortex nor the mid-brain is essential to the gonadal-light response in the ferret (Zuckerman, 1947).

There is some doubt as to whether the axons of hypothalamic neurons actually innervate the anterior lobe of the pituitary, as has been suggested by Vazquez-Lopez & Williams (1952) and Vazquez-Lopez (1953). Harris and his collaborators in several publications (Harris, 1948, 1950, 1952; Harris & Jacobsohn, 1952; Donovan & Harris, 1954) have advanced the view that the proximate stimulus for the specific activation of the secretory cells of the gland is a chemical substance which is discharged from certain hypothalamic neurons. This chemo-transmitter substance passes down the axons and diffuses into the capillary loops of the hypophyseal portal vessels whence it passes into the sinusoids of the anterior lobe (Harris & Jacobsohn, 1952). The observations of Zuckerman and his collaborators (Zuckerman, 1947, 1954; Thompson & Zuckerman, 1953, 1955), however, conflict with Harris's hypothesis of the pituitary portal system. According to these investigators (Zuckerman, 1954; and Thompson & Zuckerman, 1955) the integrity of the pituitary portal vessels is not essential for the reflex activation of the anterior pituitary, nor is it necessary to suppose that chemo-transmitters are involved in the process.

2. Temperature

It has long been assumed that high environmental temperatures are deleterious to both physiological and mental activity, and there is little doubt that, in many captive or domesticated mammals, reproductive efficiency is impaired, and that fertility is minimal during the summer months. On the other hand, Farner, Mewaldt & Irving (1953) have shown that high environmental temperature results in considerable nocturnal activity of the white-crowned sparrow, such warmth augmenting the gonad-stimulating effect of artificially long days. Birds receiving extra illumination in the cold, however, showed far greater gonadal (testicular) development than did others on warmth and diminished rations of light, although the total duration of activity in the latter group was far greater than in the former.

In the male ground-squirrel investigated by Wells & Zalesky (1940), gonadal development is not modified under laboratory conditions by extra illumination, and animals maintained at laboratory temperatures experience practically the same seasonal cycle as those under field conditions. In the wild state, testicular regression is associated with rising summer temperatures, whereas seasonal reactivation coincides with the diminishing temperatures of autumn and winter. Further proof that temperature does have a marked effect on testicular rehabilitation is given by the observation that breeding males kept at 40° F.[1] for one year remain in breeding condition throughout this period, the sexually quiescent phase of the cycle failing to appear.

Erb & Waldo (1952), reviewing the evidence relating to dairy cattle, concluded that in areas where high environmental temperatures are common during a portion of the year breeding efficiency declines during the summer. The summer depression in the production of both milk and butter-fat has likewise been known for a long time.

A. J. Marshall (1949) compared the state of the gonads and the breeding activities of several species of passerine birds after the severe winter of 1946–47, and after the very mild one of 1947–48. He found that, despite the arrested development at the primary spermatocyte stage in mid March 1947, at least three species bred at about the normal time, following a sharp rise in temperature and an increase of sunshine in April. Following the abnormally warm winter of 1947–48 three, and probably four, species bred abnormally early. He concludes that proximate factors, other than the number of hours of daylight and resultant activity, are significant in timing the breeding-seasons of birds, and that temperature and sunshine may play an important part in some species. The same author (A. J. Marshall, 1952a) finds that the post-nuptial display of the robin and the rook is partly or almost wholly extinguished in normally severe December weather, but the pre-nuptial display following this is governed by the week-to-week environmental experiences, including the behaviour reactions of the pair. Sunshine, as opposed to

[1] t° F. = ⅝(t − 32)° C.—Ed.

mere lengthening days, is a potent stimulus; excessively dull cold weather and hunger are inhibitors. A. J. Marshall (1952b) also found that the gonads of migratory passerine birds showed considerable activity on the arrival of the birds in England, and he ascribed to this activity the singing of these birds while on passage. The stomach contents showed that the birds had paused to feed on their journey north, yet, despite the advanced condition of their gonads, they had continued their flight instead of taking up territory and breeding at some suitable locality on the way. A. J. Marshall (1952c) concluded that, among non-breeding birds of the arctic, in the summer the gonad cycle of non-breeders of both sexes does not develop to its full extent because the environment does not present to the exteroceptors the appropriate stimuli, including behaviour reactions, to which each species has evolved its neuro-endocrinal response.

3. Psychological Factors

In addition to physical stimuli, many psychological factors have profound effects upon the breeding-cycle in birds and mammals. It is notorious that many species of birds and mammals, although perfectly healthy and in an apparent state of complete physiological well-being, never breed in captivity. Something is lacking in the psychological climate that either prevents breeding or fails to stimulate it.

The elaborate courtship rituals of many species of birds are a striking example of psychological stimuli that affect the breeding-cycle. In many birds the cycle—pairing, nest-building, copulation, ovulation, incubation and the rearing of young—cannot take place unless the traditional ritual is adhered to. This condition for the success of the breeding-cycle is practically universal throughout the birds, and in many species there is a very complicated sequence of activities forming the nuptial display that is essential to the successful production and rearing of offspring. So stereotyped are these sequences of events that, if they are interrupted, it is necessary for the participants to go back to the beginning and carry through the ritual in all its detail from the start, for successful breeding to occur. Examples may be found in all the families of birds from passerines to tubinares—the displays in some of the birds of paradise being perhaps the most spectacular.

Darling (1938) and Tinbergen (1951) have discussed the effect that the total numbers of individuals have on the efficiency of the breeding activity of sea-birds that nest in colonies. Darling has shown that within limits the larger the colony the higher the percentage of successful hatchings, and that there is a minimum density of numbers in the colony, below which there is little or no success in breeding. He further suggests that in some sea-birds that nest gregariously the period of egg-laying is more protracted in small colonies than in large ones, because the psychological stimulus to breeding is weaker in the small colony. In a small colony the nidifugous young appear in smaller numbers over a much longer period of time, and consequently have poorer chances of surviving attack from predators than the greater number in the large colony, whose appearance is more concentrated in time round the peak of the breeding-season.

The importance of numbers in influencing the breeding-cycle through psychological stimuli is very great in those species in which there are communal nuptial displays, such as the blackcock, ruff, fulmar, and some species of albatross. It has been suggested that the widespread reduction in numbers of the blackcock throughout the British Isles in recent years, although it is due primarily to coccidiosis, is mainly brought about by a secondary consequence of the disease, which has left a population too thinly spread to allow the necessary psychological stimuli to be built up through concentration of birds on their breeding grounds.

Darling has made his chief studies on the herring gull, but he also extended his observations to the fulmar which, as Fisher (1952) has shown, is gradually extending its breeding range in the British Isles. It is usual for this species to appear in some numbers at new nesting sites on the margins of its breeding range, and for the birds to show every apparent intention of breeding; but no eggs are laid or young reared until the colony has built up in numbers to the necessary minimum, a process that may take several years. It is suggested that an essential psychological stimulus is lacking until the number of birds in the colony attains specific minimum size.

Similar psychological stimuli necessary to successful mating and breeding are found in all families of birds: for example, the sexual displays and song of the passerine birds, the nuptial dances of the waders, ducks, tubinarine and ratite birds. Throughout the order successful breeding cannot be accomplished unless the correct psychological atmosphere is produced by the presence of birds in sufficient numbers or by the stereotyped courtship displays that lead up to and release the appropriate innate responses.

It is well known that in many species of birds the display is not confined to the breeding season, and consequently the psychological stimuli that it produces do not then immediately lead to breeding. Moreover, in many species, and particularly in those showing display activities outside the breeding season, the birds pair for life. F. H. A. Marshall (1936, 1942) suggests that display in such species serves not only for promoting an effective synchronization of the male and female sexual processes, but also for keeping the pairs together and thus securing a "marriage bond". Display outside the breeding season does not necessarily occur in species that pair for life, as is shown by some of the oceanic birds that nest in burrows. The birds are dispersed over thousands of square miles[1] of sea while they are not breeding, but the birds of each pair return to the same burrow for nesting year after year, although it is believed that the birds of a pair do not remain in company throughout the year. In this example, the restricted territory of the breeding burrow may be the bond that reunites the pair every breeding season.

It is probable that the route by which the psychological stimuli act upon the gonads is through the hypothalamus and the anterior pituitary gland. In many female birds ovulation is not spontaneous, but depends upon the appropriate releasers being presented by the sexual partner. It has been shown (Harper, 1904; Matthews, 1939) that female pigeons in solitary confinement do not usually ovulate or lay eggs, but the proximity of a companion of either sex that can participate in the nuptial display, or even the image of the bird displaying to itself in a mirror, is a sufficient stimulus to lead to the laying of eggs. It was also shown that the stimulus is visual, but not auditory or olfactory, and that the pituitary is probably stimulated to release the appropriate hormones to cause ovulation by external factors received visually and transmitted to the pituitary through the hypothalamus.

[1] 1 mile = 1.609 km.—Ed.

Little is known about the importance of psychological stimuli in the breeding-cycles of mammals, though they probably play as large a part in regulating the cycle in many species of mammals as they do in birds. Davies (1953) investigated the breeding success of a small colony of grey seals on the coast of Cardiganshire; he found the rate of reproduction to be very low and the infant mortality rate very high, although there was a total population of about fifty seals. He concluded that the smaller colonies cannot maintain themselves unaided and that their numbers must be augmented from larger colonies where the reproductive rate is much higher, perhaps because the psychological environment is more favourable.

In many species of seal the sexes are distinguished by prominent secondary sexual characters, and in some there is a characteristic harem system on the breeding grounds, whereby a dominant male makes himself the proprietor of a number of females which he guards against the attentions of rival males. In all these species there is a post-partum oestrus during which the cows are covered by the bulls, and the part that psychological stimuli may play in the breeding rhythm is unknown, if indeed any are necessary.

A harem system is also used by some ungulates, and it has been suggested, but not proved, that the stag of the red deer herding his harem of hinds, and challenging or even sparring with rival stags, provides a psychological stimulus that helps to bring his hinds into oestrus. In the roe, which is not polygamous but usually goes in small parties consisting of a buck, a doe and her fawns, there is a peculiar "courtship" habit that may well provide a psychological stimulus. In many places, particular parts of the territory of the roe form centres for the activities of the animals during the rut; these are the "racing rings", more or less circular tracks trodden out by the bucks chasing the does among the trees, round one of which the ring is often centred. Some observers (Millais, 1906) report that the doe allows the buck to chase her round and round the ring, the play sometimes lasting so long that both partners become temporarily exhausted. At the end of the chase pairing takes place, and then racing is resumed, and the whole process may be repeated many times. Buxton (1948, 1949), however, has reported a rather different pattern of behaviour. Roe watched by this observer in Scotland did not race; they walked, and only occasionally did the buck break into a slight trot when he fell behind the doe. His activity appeared to be directed to preventing the doe from leaving the area in which the ring lay, for not only did he follow her but he dodged round in front beneath her nose as if to head her off or stop her. He also shouldered her from side to side so that she was guided into a more or less circular course, and it is suggested that the ring is formed by the action of the buck in thus herding the doe on a circular track. The doe evidently enjoys this play as much as the buck for she does not attempt to break away; while a pair were watched walking the ring for half an hour the buck served the doe nine times.

4. Conclusion

From the foregoing discussion it is clear that the environment exercises a profound effect on the physiological functions of reproduction. The modes of action of the mechanisms by which changes in environmental temperature, light, food supply, and social impact affect gonadal development are, however, such complicated problems that final conclusions cannot be drawn. Surgical removal of the pituitary gland abolishes the normal seasonal cycle in many mammals and birds, and renders the gonads unable to respond to environmental factors which normally activate them. This indicates that the hypophysis is a necessary link in the chain of reactions which lead to sexual activation. It has been shown also that the gonad-stimulating potency of the hypophysis increases during the period of normal or artificial reactivation of the gonad. Thus it appears that the seasonal cycle is conditioned by certain external environmental factors which excite or inhibit the secretion of gonadotrophins by the anterior pituitary.

There is no doubt that a close physiological relationship exists between the hypothalamus and the anterior lobe of the pituitary, and the suggestion of Harris (1948), that the central nervous system regulates the activity of the adenohypophysis by means of a humoral relay through the hypophyseal portal vessels, has received considerable support. Indeed, some workers have postulated the existence of a specific "sex centre", located in the hypothalamus, which might activate the pituitary through the portal channels alluded to above. It must be pointed out, however, that Zuckerman (1954) is unyielding in his view that photoperiodic pituitary regulation is independent of the integrity of the hypophyseal portal circulation. It may thus be concluded that, whereas it is evident that gonadal function is conditioned by a variety of environmental factors external to the organism, the mode of mediation employed within the organism in such cases has not yet been satisfactorily elucidated.

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NEUROHUMORAL MECHANISMS IN REPRODUCTION

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It is clear that neural mechanisms are intimately concerned with the control of reproductive phenomena. Marshall (1936, 1942) was the first to emphasize the dependence of the breeding season in many species on environmental or "exteroceptive" factors, and he suggested that such factors exert their effects by nervous reflex stimulation of the secretion of gonadotrophic hormone (presumably follicle-stimulating hormone (FSH)) from the anterior pituitary gland. Many data have accumulated in later years to confirm and extend the views of Marshall. This work is discussed by Amoroso & Matthews elsewhere in this symposium.[1] As is well known, luteinizing hormone (LH) is released from the hypophysis of the rabbit, cat, ferret, ground-squirrel and mink, and of many birds, with resultant ovulation, as a consequence of coitus. More recent information (Everett, 1952) points also to the inclusion of the rat in this group. It may be taken as established for the rabbit, and as a strong probability for the other forms, that sexual excitement affects LH secretion by nervous reflex activation of the anterior pituitary. Copulation is said to hasten the occurrence of ovulation in cattle (Marion, Smith, Wiley & Barrett, 1950). The secretion of the third gonadotrophic hormone (luteotrophic hormone or prolactin) is stimulated in the rat by sterile coitus or prolonged suckling. In all probability these stimuli exert this effect by nervous mediation.

The data discussed below are taken mainly from publications which have appeared during the last ten years. Other evidence relating to the neural control of gonadotrophic secretion may be found in the reviews of Hinsey (1937), Hartman (1939) and Harris (1948a, 1950a).

1. Hypothalamic Stimulation and Lesions

The first direct evidence that the central nervous system is involved in the neurohumoral control of reproduction was obtained by Marshall & Verney (1936) when they found that strong electrical stimulation through the head might be followed by ovulation in the rabbit. Since that time more-localized stimuli applied to various regions in the hypothalamus have been found to result in ovulation in the rabbit (Harris, 1948b) and, conversely, more-localized lesions to result in disturbances of gonadotrophic secretion.

Recently there has been a resurgence of interest in the effect of hypothalamic lesions. Bilateral lesions posterior to the optic chiasma have been found to result in constant oestrus in the guinea-pig by Dey, Fisher, Berry & Ranson (1940) and Dey (1943), and in the rat by Hillarp (1949) and Greer (1953). The region concerned, according to the latter authors, lies anterior and ventral to the paraventricular nuclei. Barrnett & Mayer (1954), however, reported that lesions in the ventromedial nuclei produced a similar effect. It is agreed that the lesions must be bilaterally symmetrical to be effective. The above results cannot be attributed simply to the destruction of an LH-controlling "centre", for Greer (1953) found that the injection of small daily doses of progesterone was followed by a resumption of four-to-six-day vaginal cycles. Similarly, electrical stimulation of the cervix or caging with a male may also be followed by a dioestrous interval in such animals with persistent oestrus. Failure of spermatogenesis and testicular atrophy occurred in male rats with lesions in the anterior hypothalamus (Mess, 1952; McCann, 1953). Tumours of the hypothalamus may be associated with pubertas praecox in children, and recent cases of this syndrome have been reviewed by Bauer (1954). The findings in these instances conflict with those arising from experimental work in that the tumours responsible for precocious puberty mostly involved the mamillary bodies, whilst experimental lesions producing gonadal changes are usually situated in the anterior hypothalamus.

2. Pituitary Stalk

a. Nerve Supply

With the demonstration that the hypothalamus controls the gonadotrophic activities of the hypophysis, there arises the question of the anatomical route by which this regulation is exercised. The most direct path would seem to be formed by the pituitary stalk, which attaches the hypophysis to the hypothalamus.

Much time has been spent in the past in discussing the nerve supply of the anterior pituitary gland, which has been described as sympathetic, parasympathetic or hypothalamic in origin. A few sympathetic fibres possibly enter the gland at the periphery (Rasmussen, 1938) but do not seem to be of great functional significance, for cervical sympathectomy does not modify gonadotrophic activity, and the presence of an intact sympathetic system does not prevent the gonadal atrophy which follows complete pituitary stalk section. Recently Ifft (1953) followed the oestrous cycles of normal rats, and of animals after cervical sympathectomy, in constant light, constant dark, and under normal laboratory conditions. Neither vaginal-smear studies nor a differential cell count of the cell population of the pituitary glands showed any variation between the control animals and those that had been operated on. The possibility that a parasympathetic nerve supply to the hypophysis, passing via the facial nerve and carotid plexus, was concerned in the control of ovulation in the rabbit was tested by Hair & Mezen (1939) and Vogt (1942). These workers found that avulsion of the facial nerve and

[1] See Amoroso & Matthews, p. 87 of this number of the Bulletin.—Ed.

geniculate ganglion, or destruction of the greater superficial petrosal nerves, might be followed by the normal process of ovulation after coitus. The most direct route for nerve fibres supplying the anterior pituitary would be via the stalk, but these have never been convincingly demonstrated. Descriptions of nerve-like fibres have been given by Vazquez-Lopez (1949) and others. However, Green (1951) published the results of a comparative study of the hypophyses in seventy-six species of animal, ranging from lampreys to man. He states:

> . . . In none of the animals studied has an innervation of the pars distalis been found. While this does not prove that such an innervation is absent, it seems likely that there are a few if any nerve fibres in the pars distalis. That the results obtained are due to some special chemical property of the pars distalis which might theoretically inhibit impregnation (Croll, 1928) is made unlikely by the fact that small bundles of fibres to the neural lobe quite often cross a small section of pars intermedia or pars distalis and impregnate just as clearly while doing so.

Again, in 1952, Green records that a detailed impregnation of the neurohypophysis was obtained with the Bodian method, and nerve fibres were readily observed in the pars intermedia.

> Yet these preparations] have consistently failed to show nerve fibres or endings in the pars distalis proper.
>
> In preparations where glial or reticular fibres were visible in control regions . . . fibres were seen in the pars distalis which closely resembled nerve fibres.

A detailed study of the avian hypophysis by Wingstrand (1951) gives no evidence for the presence of nerve fibres in the main mass of the anterior pituitary in this form. Despite the lack of convincing evidence for a functional innervation of the pars distalis, there is clear evidence of a supply to the pars tuberalis. Green & Harris (1947) observed a moderate number of nerve fibres crossing from the hypothalamo-hypophyseal tract to the pars tuberalis. They appeared to run with the capillary loops of the portal vessels, and fibres were seen to end on these blood vessels. Cameron (1929), Hair (1938), Rasmussen (1938) and others have also described nerve fibres entering the pars tuberalis. Stutinsky (1948) has emphasized the richness of the innervation of the pars tuberalis. He noted that the nerve fibres appear to form a perivascular plexus in this part of the gland.

b. Hypophyseal Portal Blood Supply

An alternative connexion between hypothalamus and pituitary gland is provided by the hypophyseal portal vessels. Harris (1950a) has previously described these trunks, and it will suffice here to note further information which has accrued since that time. Green (1951), in his comparative study, observed the presence of portal vessels in amphibians and in all higher forms investigated. For the bird, Wingstrand (1951) noted that the portal vessels provided the only blood supply to the pars distalis. He found the median eminence to be covered by a dense network of capillaries, occupying up to 50% of the surface of this part of the brain. This plexus was drained by 15–30 vascular trunks passing to the glandular lobe of the pituitary. The capillary network was seen to be separated from the glandular layer of cells on the median eminence only by the endothelium of the vessels, by a thin reticular membrane and perhaps by a continuous glial sheath formed by the pia interna. The descriptions of Landsmeer (1951), McConnell (1953) and Xuereb, Prichard & Daniel (1954) provide a detailed analysis of the blood supply of the pituitary gland in rat and man.

c. Stalk Section

Since the nerve fibres in the pituitary stalk do not regenerate after division (Fisher, Ingram & Ranson, 1938; Magoun, Fisher & Ranson, 1939), whilst the portal vessels may quickly bridge the gap (Harris, 1950b), the technique of stalk section has been used to differentiate the functional relationship of these structures to anterior pituitary activity. In such experiments, three groups of animals may be studied: (i) animals on which sham operations have been done (intact nervous and vascular connexions in the stalk) ; (ii) animals that have been subjected to simple stalk section (nervous connexions severed and degenerate, vascular connexions severed and regenerated in a large proportion of cases) ; (iii) animals after stalk section with the placement of a plate of waxed paper, sclera or other substance between the stalk ends (both neural and vascular components of the stalk permanently interrupted). In performing such experiments it is important to pay attention to various technical details:

i. To perform the operation of stalk section by a temporal approach. Such an operative exposure gives a wide view of the whole stalk region and allows the placement of the plates with accuracy. Further, the divided stalk is left in a clear field (which is not the case with a parapharyngeal operation) and so enables clear observations to be made of the microscopic anatomy of the region after death.

ii. In order to study the vascular architecture of the stalk and gland, post mortem, it is necessary to perfuse the blood vessels of the animal with some coloured material (such as Indian ink), to fix and decalcify a block of tissue containing the skull base, pituitary gland and hypothalamus, and to cut serial sections at a thickness of 100–200 μ. after imbedding in celloidin.

There is little doubt that complete section of the pituitary stalk is followed by gonadal atrophy, and that this result is due to interruption of the vessels of the stalk which exert some specific effect on the secretory activity of anterior pituitary tissue. Such a view has recently been questioned by Thomson & Zuckerman (1953, 1954) and Zuckerman (1954a, 1954b). In a series of publications these workers describe the results obtained in two ferrets in which it was thought that complete interruption of the vessels of the pituitary stalk was compatible with an oestrous response to extra illumination in winter. Adopting the principles outlined above, Donovan & Harris (1954) have made similar studies with different results. After operation the animals were exposed to extra lighting during winter and it was found that an oestrous response occurred in four animals on which sham operations had been done, in five out of six ferrets in which simple stalk section had been performed, and in three out of fourteen animals in which waxed-paper plates had been inserted between the ends of the divided stalks. Microscopic study of serial sections through the pituitary stalk region of these animals revealed a clear correlation between the presence or absence of intact or regenerated portal vessels (in three oestrous cases regeneration had occurred around the borders of misplaced plates) and the presence or absence of the oestrous response. The suggestion put forward by Greep & Barrnett (1951), that pituitary stalk section results in disturbance of gonadotrophic secretion by producing a reduction in total blood supply and ischaemia of anterior pituitary tissue, could not be supported. Measurement of the volume of

anterior pituitary tissue revealed that the ferrets which failed to show an oestrous response after stalk section had 95% of the normal volume of anterior pituitary tissue present, whilst the animals which showed an oestrous response had only 80%. Similar results to those of Donovan & Harris (1954) have been obtained by Benoit & Assenmacher (1953) working on the drake. In this bird, as in many others, the anterior and posterior lobes of the pituitary are separated by a connective tissue septum, and the vascular and nervous components of the stalk may be individually sectioned. These workers found that cutting the nerve tract to the posterior lobe had no effect on gonadotrophic secretion, whereas section of the portal vessels, and placement of a plate of sclera between the cut ends of the vessels, resulted in testicular atrophy. They also noted a lack of correlation, after "stalk" section, between the amount of anterior pituitary tissue present and the development of the gonads.

3. Pituitary Transplants

Information has also been obtained concerning neurohumoral control of gonadotrophic secretion from studies of pituitary transplants. Such studies have indicated that gonadotrophic secretion is obtained only when the graft is placed beneath the hypothalamus and is revascularized by the portal vessels emanating from the median eminence of the brain (Harris & Jacobsohn, 1952). Pituitary tissue placed beneath the temporal lobe, or in other sites remote from the sella turcica, fails to maintain the reproductive organs. Failure of gonadotrophic secretion by these latter transplants is not due to lack of pituitary tissue. Westman & Jacobsohn (1940) measured the volume of anterior pituitary tissue after transplantation to the anterior chamber of the eye. In ten cases there was more than 30%, in five cases more than 100% and in two cases more than 200% of the figure for normal glands. In these experiments, however, there was a uniform lack of vaginal cycles associated with atrophic ovaries. More recently Fortier & Selye (1949), Cheng, Sayers, Goodman & Swinyard (1949) and Fortier (1951) have reported gonadal atrophy in hypophysectomized rats with ocular pituitary transplants.

The study of pituitary transplants (Harris & Jacobsohn, 1952) beneath the median eminence of the hypothalamus, or beneath the temporal lobe of the brain, gave incidental but interesting information with regard to the origin of (*a*) the cycles of sexual function, (*b*) the changes occurring at puberty, and (*c*) sexual differentiation of anterior pituitary tissue.

a. Cyclic Occurrence of Oestrus

Moore & Price (1932) first put forward the view that the cyclical nature of oestrus was due to humoral interplay between the ovaries and the anterior pituitary. It was supposed that an increased blood concentration of oestrogens inhibited FSH, and stimulated LH, secretion and that an increased blood level of progesterone inhibited LH. That this simple endocrine interaction is unlikely to be the whole explanation is now shown by the fact that pituitary transplants grafted on to the cortex of the temporal lobe of the brain may become well vascularized, but fail to maintain oestrous cycles. It would appear that the hypothalamus and intact hypothalamo-hypophyseal connexions are also of importance (Harris, 1950b).

b. Puberty

It has long been known that immature ovaries grafted into mature animals may show precocious function. In view of this fact it has been tentatively suggested that maturation of pituitary tissue is the factor responsible for the onset of puberty. However, pituitary glands obtained from rats a few days old are able to support adult reproductive function if grafted beneath the pituitary stalk of hypophysectomized adult female rats. These transplanted glands are brought into anatomical continuity with the hypothalamus, since they become vascularized by the hypophyseal portal system. Again, it seems likely that the hypothalamus is involved in the sudden onset of reproductive function that occurs at puberty.

c. Sexual Differentiation of Anterior Pituitary Tissue

Since ovarian tissue transplanted into normal or castrated male animals may develop ripe follicles, but rarely, if ever, shows luteinization, it has been suggested that the male anterior pituitary gland secretes less luteinizing hormone than the female gland (Pfeiffer, 1936). However, anterior pituitary tissue obtained from adult male rats and transplanted under the pituitary stalk of hypophysectomized female rats may become revascularized by the hypophyseal portal system and may maintain female sexual activity as exemplified by oestrous cycles, copulation and pregnancy on the part of the female recipient.

From the above data it would appear that anterior pituitary tissue is plastic in nature and that its activity and the hormonal pattern of its secretion depend upon the hypothalamus.

The evidence derived from experiments involving hypothalamic stimulation, hypothalamic lesions, pituitary stalk section and pituitary transplantation supports the view that gonadotrophic secretion by the anterior pituitary gland is dependent upon, and controlled by, the hypothalamus acting via the hypophyseal portal vessels of the pituitary stalk. It seems that the external environment affects the reproductive process by means of nervous reflexes originating in a wide variety of sensory end-organs. Such reflex activity would appear to be co-ordinated and integrated in the hypothalamus, some region of which is probably sensitive also to changes in the ovarian hormone concentration of the general blood stream. The eventual stimulus to pituitary secretion of gonadotrophic hormone traverses the portal vessels of the stalk, and these may be likened to a "final common path". There can be little doubt that the portal vessels exert a profound and specific effect upon anterior pituitary activity. The nature of the mechanism underlying this process is uncertain, but the most likely hypothesis is that hypothalamic nerve fibres liberate some humoral agent into the primary plexus of the vessels in the tuber cinereum and that this agent is carried by the vessels into the sinusoids of the anterior pituitary gland, where it stimulates or inhibits cellular secretion.

4. Pharmacological Data

a. Blockade of Ovulation

Evidence derived from the pharmacological blockade of reflexly induced, or spontaneous, ovulation has been put forward in support of the view that the stimulus from the hypothalamus to the anterior pituitary gland is humorally transmitted. The interpretation of many of the results obtained, however, is not yet clear. Some of this work will be briefly mentioned.

Sawyer, Markee & Hollinshead (1947) and Sawyer, Markee & Everett (1950) found that various sympatholytic agents

would block the ovulation which normally follows coitus in rabbits, provided that they were injected within 90 seconds of the mating act. Atropine was also effective, but this drug had to be administered within 30 seconds of copulation (Sawyer, Markee & Townsend, 1949). Using rats, Everett, Sawyer & Markee (1949) found that ovulation could be blocked by Dibenamine or atropine injected at a certain time during pro-oestrus. Injections made one or two hours later were ineffective. It was concluded that a neurohumoral activation of the hypophysis occurred some 10 to 12 hours before ovulation in the rat. Nembutal and other barbiturates were also effective in blocking ovulation in this animal (Everett & Sawyer, 1950). These observations have been extended to the cow by Hansel & Trimberger (1951). Administration of atropine at the beginning of oestrus delayed ovulation for from 24 to 76 hours in four of five animals.

It has been known for some time that oestrogen stimulates the release of LH in the spontaneously ovulating rat, mouse, and guinea-pig, and it was thought that the steroid acted directly upon the hypophysis. However, Dibenamine or atropine injected prior to, or as much as 20 hours after, oestrogen administration to the pregnant animal prevents ovulation in a high proportion of cases (Sawyer, Everett & Markee, 1949). Interesting results along these lines have also been obtained in the hen. Progesterone will induce ovulation in this bird (Fraps & Dury, 1943), and its action has been blocked by Dibenamine (Tienhoven, Nalbandov & Norton, 1954). Normal ovulation in the hen has been blocked with atropine, SKF-501 and Dibenamine. Nembutal failed to block ovulation in the hen when the birds were kept anaesthetized for 12 hours (Bastian & Zarrow, 1952) and has since been found to induce ovulation prematurely (Fraps & Case, 1953). In the rat made persistently oestrous with hypothalamic lesions, progesterone injections will cause a resumption of cycles, which may continue after the hormone is withheld (Greer, 1953). Such results indicate that oestrogen and progesterone act directly upon the hypothalamus.

The site of action of such drugs as atropine, Dibenamine and SKF-501 in blocking ovulation, assuming their action to be specific and not the result of stress (see below), is unknown. From the available data it is reasonable to assume that blockading drugs act on some neural mechanism in the hypothalamus or at some point in the humoral system involving the pituitary portal vessels. For example, Markee and his colleagues suggest that atropine acts, in this respect, by blocking synaptic transmission in the hypothalamus and that sympatholytic drugs act by blocking an adrenergic mechanism at the portal vessel-pituitary level. The fact that barbiturates, in low doses, are effective in blocking ovulation in the rat points to the involvement of the hypothalamus.

b. Excitation of Ovulation

Further data pertaining to the site of action of the blockading drugs, and to the nature of the hypothetical humoral transmitter in the portal vessels, have been obtained from experiments involving the local administration of various agents to different regions of the hypothalamus or pituitary gland. Markee, Sawyer & Hollinshead (1948) exposed the pituitary glands of 51 rabbits by the parapharyngeal approach for the injection of various concentrations of adrenaline. Eleven rabbits ovulated, of which four (of seventeen) had received 1,200 μg. of adrenaline hydrochloride, five (of ten) had received 120 μg., and one in each of two groups of ten 12 μg. and 2.4 μg. of adrenaline hydrochloride respectively. The amine was injected in three portions of 0.04 ml. over 30 minutes. An acetylcholine–eserine mixture, similarly applied, gave negative results. Injection of adrenaline or noradrenaline into the third ventricle has also evoked ovulation in the rabbit, and this effect appears to be blocked by the adrenolytic drug SKF-501 (Sawyer, 1952). This worker later found that 0.25–0.5 mg. of histamine injected into the third ventricle under local anaesthesia produced ovulation in one of seven animals, whilst with general (Nembutal) anaesthesia six of seven rabbits ovulated (Sawyer, 1954).

Studies on the effect of adrenergic and other agents injected into the hypothalamus have since been made by Donovan & Harris (unpublished). The tuber cinereum was approached from above by a needle inserted vertically in the mid-line, and various solutions were injected over periods of up to 100 minutes at a rate of 0.0002 ml./minute. It was found that 120 μg. of adrenaline or noradrenaline bitartrate produced ovulation when injected over 100 minutes into the hypothalamus. Ovulation occurred in six of twelve rabbits injected with noradrenaline bitartrate and in seven of sixteen rabbits given adrenaline bitartrate. However, when the pH of the solution was adjusted to neutrality with sodium bicarbonate, the percentage of positive responses was greatly reduced, even when the amount of amine was increased to 150 μg. to offset an increased rate of decomposition. Thus five animals injected with 150 μg. of noradrenaline in neutral solution uniformly failed to ovulate, and only one of eight rabbits injected with 150 μg. of adrenaline bitartrate of neutral pH ovulated. As control injections of physiological saline made into the hypothalamus were followed by ovulation in a similar proportion of experiments, the positive results from the adrenaline injections are of doubtful significance. Injections of adrenaline or noradrenaline made directly into the pituitary gland yielded ovulation only with acid adrenaline solution. The conclusion drawn from these results was that adrenaline and noradrenaline are probably not concerned with the humoral stimulation of gonadotrophic secretion.

c. Evidence Regarding an Adrenergic Transmitter

The evidence in favour of an adrenergic transmitter being involved in the hypophyseal portal system as an excitant to gonadotrophic secretion is weaker than it first appears. Three main points may be mentioned.

Firstly, noradrenaline has been found to be present in the hypothalamus (Vogt, 1953, 1954) and is especially concentrated in the medial areas of this structure. On this ground it might be supposed to be related to the nerve tracts and portal vessels involved in regulating gonadotrophic secretion. However, Vogt showed that similar concentrations were present in other regions of the brain stem. Further, noradrenaline is less active in exciting anterior pituitary tissue, when applied locally, than adrenaline—and this latter compound is present in such areas of the brain in only very small amounts.

Secondly, the sympatholytic drugs employed in blocking ovulation are not solely adrenolytic in action. Thus Dibenamine possesses some antihistaminic activity (Nickerson, 1949), and is said to be one of the most powerful antagonists of 5-hydroxytryptamine (Erspamer & Sala, 1954). Also, the sympatholytic effect takes time to develop because of the in-vivo formation of active intermediary compounds (Nickerson, 1949), whilst Dibenamine will block ovulation in the rabbit only if injected within one-and-a-half minutes of

copulation. The effect appears to be almost instantaneous. Timiras (1952) suggested that LH release in the rat was blocked, not by a sympatholytic effect of Dibenamine, but by a stressing effect of the drug. To support this view, evidence was presented that injections of adrenaline, eserine or formalin, or spinal cord resection, blocked pituitary hormone release in pregnant rats after oestrogen injection in about 40% of cases. However, Everett (1952) pointed out that dibenzylaminoethanol and Banthine, which seem to be powerful stressing agents, fail to prevent pituitary activation.

Thirdly, the amount of adrenaline necessary to produce ovulation after local application of the drug to the anterior pituitary is high. If the solution of the acid adrenaline salt is first carefully neutralized, the percentage of positive responses is much reduced, a fact which cannot be explained by the increased decomposition rate of adrenaline in neutral solution.

d. Other Possibilities

Other substances, one of which may act as the possible transmitter in the portal vessels, may be cited. Serotonin, Substance P, histamine, and acetylcholine are all found in high concentration in the hypothalamus. Since some effects of serotonin are blocked by Dibenamine and atropine, this substance has properties which would fit some observations. Another substance suggested for the role of transmitter is the neurosecretory material of the supraoptic and paraventricular nuclei. Morphological evidence indicates that this material passes into the portal vessels, but physiological evidence so far allows the substance only antidiuretic, oxytocic and vasopressor properties. However, the findings that lesions of the supraoptic and paraventricular nuclei are not invariably associated with gonadal atrophy would argue against this view.

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BIOLOGY OF THE GONADOTROPHINS

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1 Chorionic gonadotrophin (CG)
2 Pregnant mare serum gonadotrophin (PMS)
3 Follicle-stimulating hormone (FSH)
4 Luteinizing hormone (LH)
5 Luteotrophic hormone (LTH)
References

For the purpose of this paper the various gonadotrophins have been divided into the following groups according to their origin and major site of occurrence.

Extra-pituitary origin
- Chorionic gonadotrophin (CG): found in human placenta, serum and human pregnancy urine.
- Pregnant mare serum gonadotrophin (PMS): found in the uterus and blood during pregnancy in the mare.

Pituitary origin
- Follicle-stimulating hormone (FSH): found in the anterior pituitary and in human urine, increasing following oestrogen deficiency.
- Luteinizing hormone (LH): found in the anterior pituitary and synonymous with interstitial cell stimulating hormone (ICSH)
- Luteotrophic hormone (LTH): found in the anterior pituitary and synonymous with prolactin.

Many detailed reviews on this subject have been published (Friedman, 1941; Hisaw, 1947; Evans & Simpson, 1950; Diczfalusy, 1953), so that the present contribution will attempt to bring together and emphasize some of the newer trends in research in this field of biology.

1. Chorionic Gonadotrophin (CG)

The occurrence of gonadotrophic activity in human pregnancy urine was originally described by Aschheim & Zondek (1928). The early confusion over the possible source of this hormone has now been settled, with the acceptance that the cytotrophoblast is the site of production. Intraocular grafts of chorionic tissue (Kido, 1937) or chorionic villi growing in tissue culture may synthesize the hormone (Jones, Gey & Gey, 1943). Chorionic gonadotrophin is produced during pregnancy only in the human and in some of the higher apes. It is excreted in the urine, in amounts readily detectable by biological assay, two to three weeks after fertilization, and abruptly increases to a peak of from 40,000 to 400,000 i.u./24 hr. between 50 and 70 days after the last menstrual period (Lyon, Simpson & Evans, 1953). Thereafter there is a moderately steep decline in excretion although the level remains elevated until the termination of pregnancy. The gonadotrophin found in the blood of pregnant women is probably CG.

Chorionic gonadotrophin, when tested in the hypophysectomized rat, induces only stimulation of interstitial cells and repair of deficiency wheel cells in the female, and does not cause follicular development. Ovarian hypertrophy is slight, but uterine stimulation due to oestrogen production may be marked. In the male, repair and stimulation of interstitial cells occur with resulting androgen production. As a result, spermatogenic tissue may be maintained (Evans & Simpson, 1950; Liu & Noble, 1939). The effects in the intact rat are in sharp contrast to those in the hypophysectomized rat, due to a synergistic action with pituitary gonadotrophins. Thus, in the female, CG injection is followed by ovarian hypertrophy due to follicular development and corpora lutea formation. The associated hyperaemia and ovulation in immature animals serve as indices for pregnancy diagnostic tests. In the male, all testicular elements are stimulated, with a resulting hypertrophy of the testes and secondary sex organs. In species such as the guinea-pig, no synergistic action takes place with CG, and the response is similar to that occurring in the hypophysectomized rat (Leonard, 1934). Despite the marked changes caused in experimental animals by CG injection, the effects on human beings are less dramatic. Probably difference in species response, purity of the hormone preparation, and dosage or route of administration, are important factors influencing the effects in man. Intravenous therapy with purified CG has, however, been said to induce some follicular development and uterine bleeding in women (Claesson, Högberg, Rosenberg & Westman, 1948).

Recently, Lyon *et al.* (1953) have added to earlier evidence that human pregnancy urine may also contain FSH activity, presumably of pituitary origin. Whereas alcohol precipitates of urine obtained up to the 33rd day of pregnancy showed only the typical CG effects, later specimens caused, in addition, stimulation of follicular growth in hypophysectomized animals. This would suggest that certain extracts presumed to contain only CG may in reality contain small amounts of FSH and be able to exert a more complete gonadotrophic action. There is little knowledge concerning any mechanism of control over CG production by the placenta. Apparently it is an inherent metabolic product of the trophoblast, since it may be produced in large quantities and isolated from chorionepithelioma, certain testicular tumours and metastatic deposits from such neoplasms (Hamburger, 1947).

Synergistic Action

True synergism of CG and pituitary preparations was demonstrated when Leonard (1932) showed that prolan (CG), when combined with a water-soluble fraction of anterior pituitary, produced ovaries of an average weight 142% greater than the sum of the weights obtained by separate assay. Despite the increasing purity of gonadotrophic preparations the augmenting action may still be demonstrated. Using pure pituitary FSH, according to physico-chemical standards, Simpson, Li & Evans (1951) showed that CG and FSH had a definite synergistic relationship in hypophysectomized animals. About one-fifth of the dose of either substance that is effective alone can be detected by the combination with adequate amounts of the other. Steelman & Pohley (1953) have recently standardized this reaction so that it can be used for FSH assay. It is of interest to note that, in intact animals, non-specific augmentation of CG may be induced by prostigmine (Fluhmann, 1952), and by various colloids (Pedersen-Bjergaard & Tønnesen, 1950).

2. Pregnant Mare Serum Gonadotrophin (PMS)

In 1930 Cole & Hart demonstrated the presence of a potent gonadotrophin in the serum of the pregnant mare, which reaches a peak at about the 80th day of pregnancy. Initially it was considered to originate in the pituitary or chorion (Catchpole & Lyons, 1934), but Cole & Goss in 1943 demonstrated that the gonadotrophin was produced by specialized structures of the endometrium, called endometrial cups. These have been extensively studied by Clegg, Boda & Cole (1954). PMS is not excreted in the urine in appreciable quantity, and may remain in the blood and act for prolonged periods even after a single injection (Catchpole, Cole & Pearson, 1935).

The effect of PMS injections on hypophysectomized animals is in striking contrast to that of CG. A marked increase in ovarian weight takes place, proportional to the dose administered. Follicular growth, interstitial cell stimulation and theca luteinization occur, and under certain conditions ovulation is followed by corpus luteum formation. Oestrogen production is increased. If intact animals are used, the actions of injected PMS are the same but of greater magnitude (Williams, 1944a). Large doses of the hormone may cause superovulation and, if the ova are fertilized, superfecundation results (Cole, 1937).

Although the action of PMS is usually ascribed to a single hormone, it is curious to note that, after suitable treatment with agents such as periodic acid (Whitten, 1950) or extracts of the plant *Lithospermum* (Noble, Plunkett & Graham, 1954), the hormone may then induce effects predominantly of follicle stimulation. In the hypophysectomized male, stimulation of the interstitial cells of the testes is striking, and results in a marked hypertrophy of the seminal vesicles and prostate. Development of spermatozoa also occurs (Liu & Noble, 1939).

PMS has failed to become of importance in clinical medicine because of its failure to stimulate the human gonad as it does that of the lower animals, and because of its marked antigenic properties. It has been found, however, to augment the effect of crystalline CG and to cause follicular rupture and corpus luteum formation in the human (Claesson *et al.* 1948).

3. Follicle-Stimulating Hormone (FSH)

Since 1949 FSH has been prepared in a physico-chemically pure material for biological studies (Li, Simpson & Evans, 1949). From a comparison of the chemical and antigenic properties of hog and sheep pituitary FSH, it appears likely, however, that different forms of the hormone may exist (van Dyke, P'an & Shedlovsky, 1950). Small amounts of gonadotrophin, considered to be primarily pituitary FSH, are excreted in the urine, and these amounts increase by about 5 to 20 times after removal of the ovaries or cessation of their function.

Earlier reports on the action of pituitary FSH are controversial and complicated by the occurrence of traces of LH contaminating the extracts. The presence of LH is now known to affect markedly both the quantitative and qualitative effects of the hormone. Studies using intact animals are therefore of little significance.

Reinvestigating the biological effects of pure sheep FSH, Li *et al.* (1949) have shown that in the hypophysectomized female rat it causes only slight ovarian follicular growth, and no repair of interstitial cells. Full oestrous changes did not occur in the uterus. Combining this preparation with very small amounts of CG allowed a full ovarian response with oestrus to be obtained. Pure FSH given to the immature hypophysectomized male, and started immediately after hypophysectomy, was (depending upon dose) capable of maintaining testicular weight and causing the development of motile spermatozoa. The interstitial cells were " partially maintained ", but the accessory organs were the size of the untreated hypophysectomized controls. Similar studies have shown that purified hog FSH does little to stimulate follicle growth and oestrogen production in hypophysectomized rats unless very large doses are used. Such high doses probably contained traces of LH (van Dyke *et al.* 1950).

4. Luteinizing Hormone (LH)

Highly purified LH has been obtained and its biological effects studied by a number of groups of workers since 1939 (Fevold, 1939; Fraenkel-Conrat, Li, Simpson & Evans, 1940; Shedlovsky, 1940). Although their endocrine actions are identical, LH from sheep and swine pituitaries possess different immunological specificity. In the immature female hypophysectomized rat the most sensitive and specific test is the ability of LH, injected intraperitoneally, to repair the degenerated interstitial cells of the ovary. The effect on such deficiency cells may be readily demonstrated histologically, and with pure preparations no follicle stimulation takes place. When FSH and LH are used synergistically, follicular maturation and luteinization take place. In the male, LH stimulates the interstitial cells of the testes, with resulting androgen liberation. The extent of male sex hormone production may be readily measured by weight changes in the seminal vesicles, or particularly in the ventral lobe of the prostate in the rat. Spermatogenesis may be maintained, but this may be secondary to androgen production. In both the male and the female, LH is chiefly responsible for the production of the respective sex hormones. Since both FSH and LH do not physiologically act as single entities at separate times, their individual actions are chiefly of academic and chemical interest. FSH secretion may be envisaged as occurring initially in amounts sufficient to start follicular growth in the ovary. Under the increasing influence of LH, follicular maturation and oestrogen production occur. Ovulation is then induced, followed by corpus luteum formation. It is of interest that, at least in the hypophysectomized immature rat, oestrogens may act like FSH and maintain follicular development (Williams, 1944b, 1944c).

Control of FSH and LH

It would appear that the secretions of FSH and LH by the anterior pituitary are under separate control, effected mainly by direct action of the sex hormones. The gonadotrophin content of the human pituitary, which is low in childhood, increases greatly during the reproductive years. Similar concentrations of FSH and LH are found in both sexes. In women after the menopause the pituitary FSH content is disproportionately increased (Henderson & Rowlands, 1938; Noble, Rowlands, Warwick & Williams, 1939; Bahn, Lorenz, Bennett & Albert, 1953). Conversely, the administration of 10 mg. of oestradiol daily to women who have passed the menopause reduced the total gonadotrophin content of the pituitary (Rowlands & Sharpey-Schafer, 1940). Changes in urinary FSH appear to parallel the hormone content of the pituitary, the increased excretion in the post-menopausal state being reduced by large doses of exogenous oestrogen. Small

doses of oestrogen may, however, cause pituitary stimulation, and in women single injections of oestrogen given prior to ovulation may cause a release of gonadotrophin (Brown, Bradbury & Jungck, 1953). Evidence that progesterone may affect pituitary gonadotrophin is described in experiments on spayed monkeys. Although 100 μg. of oestradiol were found to reduce the pituitary gonadotrophin content, 10 μg. were ineffective unless combined with 2 mg. of progesterone (Salhanick, Hisaw & Zarrow, 1952). Progesterone alone, on the other hand, may cause a refractoriness of the gonads in their response to gonadotrophin (Slechta, Chang & Pincus, 1954). Numerous papers have emphasized the action of oestrogens on pituitary gonadotrophin in experimental animals (Noble & Collip, 1941; Hellbaum & Greep, 1946; Finerty & Meyer, 1950). Of recent interest is the suggestion that a reciprocal relationship may exist between certain pituitary hormones, particularly adrenocorticotrophic hormone (ACTH) and the gonadotrophins. The administration of cortisone or ACTH to man frequently results in an elevation of urinary FSH (Smith, 1951; Maddock, Chase & Nelson, 1953; Sohval & Soffer, 1951). In five patients with varying degrees of hypopituitarism and showing no detectable FSH in the urine, treatment with a maintenance dose of cortisone was followed in two cases with normal urinary FSH excretion (Plunkett & Hamblen, unpublished data, 1954). During pregnancy in the human, the pituitary apparently has a low content of FSH and LH (Philipp, 1930; Vozza, 1932; Saxton & Loeb, 1937; Bruner, 1951). On the other hand, in the mouse, rat and other species, where the placental production of gonadotrophins is of a lesser degree, the pituitary at certain stages of pregnancy may show increases, chiefly in its LH content (Ladman & Runner, 1953).

5. Luteotrophic Hormone (LTH)

The relationship of prolactin to the gonads was first demonstrated by Riddle & Bates (1933), when they found that the hormone reduced the testicular weight of pigeons. Interruption of the oestrous cycles of the mouse (Dresel, 1935), and rat (Lahr & Riddle, 1936), was associated with ovaries containing numerous large corpora lutea. Deciduoma may be produced in hypophysectomized rats treated with either prolactin or ACTH (Evans, Simpson, Lyons & Turpeinen, 1941).

In 1941 Astwood introduced the term luteotrophin to describe the effect of prolactin on the gonads, and postulated that pituitary LH completes follicular maturation and ovulation and formation of corpora lutea. At this stage LTH is responsible for maintenance of the corpus luteum and the secretion of progesterone. Regression of the corpus luteum may occur because of a self-limiting factor in the corpus itself, since there is no evidence of a cyclic change in pituitary luteotrophin production.

Assay of LTH may be carried out in immature female rats in which corpora lutea formation had occurred owing to injected CG. The rat is then given a daily maintenance dose of oestrogen for the duration of the assay. When vaginal cornification has taken place, the rat is hypophysectomized. Luteotrophin injected into such an animal causes maintenance and function of the corpora lutea and prevents vaginal oestrus due to injected oestrogen, with consequent vaginal mucification (Astwood, 1941). Another method is based on the ability of LTH to cause placentoma formation in a suitably primed hypophysectomized rat (Evans, Simpson & Lyons, 1941).

It should be appreciated that LTH is responsible for the onset of lactation in mammals following parturition. For a time it was believed that the high level of oestrogen during pregnancy inhibited the formation or release of LTH from the pituitary. Following the drop in oestrogen at parturition, LTH was thought to be released to cause the onset of lactation (Nelson, 1936). The control of LTH would now appear to be more complex, since there is evidence that oestrogens and pregnancy either increase or do not alter the blood or pituitary level of LTH, and that the inhibition of lactation by the sex steroids is probably a direct effect on the mammary tissue (Meites & Sgouris, 1954).

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CHEMISTRY OF THE GONADOTROPHINS

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1 Luteinizing hormone (LH)
2 Follicle-stimulating hormone (FSH)
3 Chorionic gonadotrophin (CG)
4 Pregnant mare serum gonadotrophin (PMS)
5 Concluding remarks
Addendum
References

At the present time, a number of substances are recognized as possessing gonadotrophic activity in terms of the bio-assay methods discussed by Emmens in another paper in this number of the Bulletin.[1] Four of these are fairly well characterized chemically and biologically, and their existence as definite entities is generally accepted. Two of these substances are of pituitary origin, being secreted by the anterior lobe, the other two are of extrapituitary origin. The hypophyseal gonadotrophins are (i) luteinizing hormone (LH), metakentrin or interstitial cell stimulating hormone (ICSH); (ii) follicle-stimulating hormone (FSH) or thylakentrin. The gonadotrophins of extrapituitary origin are (i) chorionic gonadotrophin (CG); (ii) pregnant mare serum gonadotrophin (PMS).

Gonadotrophic activity has also been detected in the urine of women after the menopause, and of normal men. The substances responsible may or may not be identical with certain of the hypophyseal gonadotrophins. Up to the present, the very small quantities available have precluded their characterization or comparison with other gonadotrophins. For a recent study of urinary gonadotrophins see Crooke, Butt, Ingram & Romanchuck (1954).

1. Luteinizing Hormone (LH)

Fevold, Hisaw & Leonard (1931) first provided evidence that the pituitary gland produces at least two substances, both of which are necessary for the normal function of the reproductive cycle in the female. Fevold and his collaborators later achieved a partial fractionation of the two active principles. The two factors were called luteinizing hormone (LH) and follicle-stimulating hormone (FSH) respectively. Their biological effects and the methods of bio-assay are discussed elsewhere in this Bulletin. Independently, H. M. Evans and his collaborators had been studying the hypophyseal factor responsible for stimulation of the interstitial cells of the testis in the male, which they had called interstitial cell stimulating hormone (ICSH). Later work showed that LH and ICSH were identical. In 1940, Li, Simpson & Evans working with sheep glands, and Shedlovsky, Rothen, Greep, van Dyke & Chow working with pig glands, independently isolated protein preparations with high luteinizing activity that satisfied the current criteria of protein homogeneity. Relatively little chemical work has been carried out on these preparations since their isolation.

i. Sources and extraction

Several workers have compared the LH potencies of pituitary glands from various animal species. The work of Fevold (1939) and West & Fevold (1940) suggests the following order: sheep > pig > horse > ox. Sheep and pig glands are the best sources available commercially. Li & Evans (1948) state that frozen glands may be stored at −15° C. for over a year without loss of biological activity.

A variety of solvents have been used for the extraction of LH from pituitary glands. Fevold (1939; Fevold, Lee, Hisaw & Cohn, 1940) used 2% aqueous pyridine or ammonia for the purpose. Li *et al.* (1940) used 40% aqueous ethanol for sheep glands, while Shedlovsky *et al.* (1940) used 2% sodium chloride for pig glands. Both the latter methods extracted FSH simultaneously with LH, so that a single extract could be used for the preparation of both hormones.

ii. Purification

Li *et al.* (1940) fractionated their extracts by precipitation with alcohol and acetone, followed by fractional precipitation with ammonium sulphate. LH, unlike FSH, is insoluble in half-saturated ammonium sulphate solution and, by repeated precipitation, the last traces of FSH can be removed. Shedlovsky *et al.* (1940) carried out their fractionation entirely by ammonium sulphate precipitation at different pH values. They also relied on the high solubility of FSH in strong salt solutions for the removal of this hormone from the LH preparations. Both preparations were finally dialysed free from salt and freeze-dried.

[1] See Emmens, p. 135 of this number of the Bulletin.—Ed.

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iii. Evidence of homogeneity

Li *et al.* (1940) subjected their preparation to electrophoretic and ultracentrifugal examination, and also determined solubility curves in the presence of varying amounts of solid phase. In all these tests, their preparation behaved as a single homogeneous protein. Shedlovsky *et al.* (1940) examined their preparation electrophoretically at pH 4.2, 5.45 and 7.85 and bio-assayed the material from different levels in the electrophoretic cell. Similarly they bio-assayed the supernatant and sedimented protein after ultracentrifugation, and the protein in solution in the presence and absence of solid phase. In all these tests, the biological activity accompanied the single protein component. Although the evidence presented for the homogeneity of sheep and pig LH appears very convincing, it is very desirable that these preparations should be re-examined by modern methods of protein fractionation such as zone electrophoresis and chromatography.[2]

iv. Chemical and physico-chemical properties

The isolation of LH from pig and sheep pituitary glands revealed the fact that there was a striking species difference. Table I shows that the two preparations differed in molecular weight, in isoelectric point and in chemical composition. Chow (1942) has also shown that the two proteins are immunologically different.

TABLE I. PROPERTIES OF SHEEP AND PIG LUTEINIZING HORMONES

Property	Sheep	Pig
Molecular weight . .	40,000	100,000
Isoelectric point (pH) .	4.6	7.45
Tryptophan content (%)	1.0	3.8
Mannose content (%) .	4.5	2.8
Hexosamine content (%)	5.8	2.2

Both preparations are glycoproteins, as are all the other gonadotrophins. No complete amino-acid analyses of LH preparations have so far appeared. Fraenkel-Conrat, Simpson & Evans (1939, 1940) found that, under certain conditions, sheep LH was reduced by cysteine, with considerable loss of biological activity. They ascribed this to reduction of disulphide linkages, with consequent loss of the specific configuration of the protein. Li *et al.* (1940) have concluded, as a result of acetylation experiments with ketene, that free amino groups are essential for the biological activity of sheep LH. Investigations of the action of proteolytic enzymes on LH have been conflicting, mainly because of the use of impure enzyme and hormone preparations. The most complete study is that of Chow, Greep & van Dyke (1939), who found that the biological potency of pig LH is destroyed by crystalline chymotrypsin, and by trypsin, but is only partially affected by crystalline carboxypeptidase or pepsin. In the case of pepsin, digestion of up to 80% of the protein still left appreciable LH activity. These results were confirmed by Abramowitz & Hisaw (1939), who also showed that, unlike FSH, LH was unaffected by crude carbohydrase preparations. These results suggest that, in the light of recent work with the adrenocorticotrophic hormone (ACTH), the chemistry of LH should be reinvestigated.

[2] See symposium on Chromatography, *Brit. med. Bull.* 1954, Vol. 10, No. 3.—ED.

2. Follicle-Stimulating Hormone (FSH)

i. Sources and extraction

The work of Fevold (1939) suggests that horse pituitaries are by far the best source of FSH, being approximately ten times as rich as the next best, i.e. pig glands. Sheep pituitaries are comparable to pig, while beef glands have a low content of this hormone.

Various solvents have been used for the extraction of FSH. Fevold and his collaborators used aqueous ammonia or pyridine. Li, Simpson & Evans (1949) used calcium hydroxide solution, and these workers have also used 40% aqueous ethanol as in their LH extraction method. McShan, Kagawa & Meyer (1954) have recently suggested the use of saturated sodium chloride at 70° C. for the extraction of the hormone.

ii. Purification

Several workers have prepared purified FSH fractions from pituitary gland extracts, but the only preparation that has satisfied physico-chemical and biological criteria of homogeneity is that isolated by Li *et al.* (1949; see also Li, 1949; Li & Pedersen, 1952) from sheep glands. Calcium hydroxide solution extracts were fractionated with varying concentrations of ammonium sulphate, by making use of the relatively high solubility of the hormone in concentrated salt solutions. The final product was dialysed and freeze-dried. McShan *et al.* (1954) fractionated their saturated sodium chloride extracts by isoelectric precipitation and adsorption of the hormone on the anion-exchange resin Amberlite XE 59. The active fraction was eluted with sodium acetate solution, dialysed and freeze-dried. This preparation was electrophoretically not homogeneous but was free of luteinizing, thyrotrophic, lactogenic and adrenocorticotrophic activities. The simplicity of the preparation would appear to make it very suitable for the preparation of FSH for biological studies.

iii. Evidence of homogeneity

Li & Pedersen (1952) examined the preparation of Li *et al.* (1949) electrophoretically at seven different pH values and concluded that it was homogeneous. They also examined several similar preparations in the ultracentrifuge. Some contained a small amount of impurity, but one was homogeneous. Diffusion studies were also consistent with the presence of only one component.

iv. Chemical and physico-chemical properties

The physico-chemical properties of the preparation studied by Li and Pedersen are given in Table II. The amino-acid and carbohydrate analyses are given in Table III. It will be seen that the carbohydrate content is lower than that of the other gonadotrophins, with a hexose: hexosamine ratio of approximately unity. van Dyke, P'an & Shedlovsky (1950) have prepared an FSH fraction of an estimated 80–85% purity from pig glands. It had similar properties to, and the same isoelectric point as, the sheep FSH of Li *et al.* (1949), but was immunologically distinct. The carbohydrate moiety of pig FSH appears to be mannose. Fraenkel-Conrat *et al.* (1939) showed that the biological activity of FSH, like that of LH, was reduced by treatment with cysteine, and concluded that, in both cases, the integrity of —S—S— linkages was necessary for full biological activity. Chow *et al* (1939) have reported that FSH is inactivated by trypsin and partially inactivated by chymotrypsin. They found that FSH was inactivated by peptic hydrolysis, but Li (1950) found that the

TABLE II. PHYSICO-CHEMICAL PROPERTIES OF FOLLICLE-STIMULATING HORMONE

Sedimentation constant (*S*)	4.7
Diffusion constant	6.0×10^{-7}
Molecular weight	67,000
Isoelectric point (pH)	4.5

biological activity of his FSH preparation was retained after 65% of the protein had been digested. He also found that the biological activity of such digests was dialysable, whereas the undigested and non-dialysable fraction was inactive. This work suggests that either the biological activity resides in a small portion of the protein molecule or alternatively that the activity is due to a very small amount of a highly active component, as has proved to be the case with ACTH.

3. Chorionic Gonadotrophin (CG)

The presence of a substance in human pregnancy urine with a luteinizing gonadotrophic activity was first demonstrated by Aschheim & Zondek (1927). Browne & Venning (1936) showed that this factor reaches its maximum concentration in the urine between the 60th and 80th days of pregnancy. There is now substantial evidence to indicate that CG is formed primarily in the placenta. No homogeneous preparation of CG has been isolated up to the present time.

i. Sources and extraction

The only known source is human urine. Pregnancy urine is the most usual source, but the gonadotrophin excreted in relatively large amounts by patients with chorionepitheliomas is probably identical with the pregnancy gonadotrophin.

The earlier workers concentrated the hormone from pregnancy urine by adsorbing it on benzoic acid which was precipitated in the urine. This process was first suggested by Katzman & Doisy (1932). In 1943, Katzman, Godfrid, Cain & Doisy adsorbed the hormone from the urine directly on Permutit (an artificial aluminosilicate) used in a chromatographic column. After washing with water, 76% ethanol, and 76% ethanol containing 10% ammonium acetate, the biological activity was eluted with 38% ethanol containing 10% ammonium acetate and was recovered from the eluate by increasing the ethanol concentration. Katzman *et al.* (1943) obtained very active preparations directly by this method. This work has been confirmed in my laboratory. The substitution of a synthetic zeolite, Decalso, for Permutit gave more reproducible results. The method of Katzman *et al.* (1943) is, however, undoubtedly the one of choice, as preparations of up to 6,000–8,000 i.u./mg. can readily be obtained directly from pregnancy urine. CG may also be adsorbed on kaolin and subsequently eluted with 0.1 N-NaOH (Scott, 1940; Dekanski, 1949; Loraine, 1950). The hormone may be precipitated with acetone from the eluate. CG has also been concentrated for bio-assay by ultrafiltration of urine.

TABLE III. CHEMICAL COMPOSITION OF FOLLICLE-STIMULATING HORMONE

Constituent	g./100 g. protein
Arginine	5.3
Aspartic acid	9.3
Cysteine	4.3
Glutamic acid	13.4
Histidine	3.7
Isoleucine	3.3
Leucine	9.2
Lysine	11.1
Methionine	1.0
Phenylalanine	5.8
Proline	5.2
Threonine	4.7
Tyrosine	3.8
Valine	5.8
Hexose	1.2
Hexosamine	1.5

ii. Purification

The best preparations of CG so far described are those of Gurin, Bachman & Wilson (1939, 1940), who assayed 6,000 i.u./mg., and of Katzman *et al.* (1943) assaying up to 8,500 i.u./mg. The claim of Claesson, Högberg, Rosenberg & Westman (1948) that they have prepared crystalline CG with an activity of 6,000–8,000 i.u./mg. has not been adequately confirmed. Morris & Morris (unpublished work, 1954) isolated material of activity up to 12,000 i.u./mg. from the urine of a patient with chorionepithelioma by a modification of the method of Katzman *et al.* (1943). Gurin *et al.* (1939) used the benzoic acid adsorption method, followed by ethanol precipitation and removal of inert proteins by denaturation with chloroform. Katzman *et al.* (1943) and Morris & Morris (unpublished work, 1954) obtained preparations of similar potency directly by ethanol precipitation of the chromatographic eluates.

iii. Evidence of homogeneity

Gurin *et al.* (1939, 1940) and Lundgren, Gurin, Bachman & Wilson (1942) subjected their material to electrophoresis at pH 7.0 and to ultracentrifugation and diffusion experiments. They concluded that the material was homogeneous. Their data indicated a molecular weight of 100,000. Katzman *et al.* (1943) gave no evidence of homogeneity. Morris & Morris (unpublished work, 1954) subjected their material to electrophoresis at five pH values without detecting any inhomogeneity. It was, however, inhomogeneous by sedimentation and solubility studies. In view of the high potency of this material, it seems unlikely that pure CG has been prepared so far.

iv. Chemical and physico-chemical properties

CG is a glycoprotein with high carbohydrate content. Some analytical data of the preparations of Gurin *et al.* (1939) and Morris & Morris (unpublished work, 1954) are given in Table IV, although it must be emphasized that the latter preparation was not homogeneous. Gurin *et al.* (1940) identified the hexose as galactose. Both preparations had a hexose : hexosamine ratio of 2 : 1, in contrast to LH and FSH where the ratio is approximately 1 : 1. Gurin *et al.* (1939) identified tyrosine, histidine, arginine and tryptophan in their preparation. Cysteine was absent.

CG is stable in the dry state. Morris & Morris (unpublished work, 1954) found that their preparation was stable in 0.2% aqueous solution for 18 hours at 0° C. from pH 1.0 to pH 13 and for 8 days at 0° C. between pH 3 and pH 11. This is in contrast to some earlier reports. It was inactivated by benzoyl chloride, phenyl *iso*cyanate, dinitrofluorobenzene, sodium hypobromite, Folin's phenol reagent and diazobenzenesulphonate. Nitrous acid did not affect the activity,

TABLE IV. CHEMICAL PROPERTIES OF PREPARATIONS OF CHORIONIC GONADOTROPHIN

	Gurin, Bachman & Wilson (1940)	Morris & Morris (unpublished work, 1954)
Potency (i.u./mg.)	6,000–8,000	12,000
Galactose (%)	10.7	12.2
Hexosamine (%)	5.2	4.9
Tyrosine (%)	present	5.1
Arginine (%)	present	3.84
Tryptophan (%)	present	0.8
		Glycine, alanine, valine, leucine, aspartic acid, glutamic acid, serine, threonine, cystine, lysine, phenylalanine, proline, isoleucine and methionine were also identified qualitatively

which was, however, somewhat reduced by formaldehyde. Abramowitz & Hisaw (1939) found that CG was rapidly inactivated by trypsin and chymotrypsin and slowly inactivated by ptyalin. The hormone is, however, remarkably stable to pepsin (Wiesner & Marshall, 1931).

4. Pregnant Mare Serum Gonadotrophin (PMS)

The presence of a gonadotrophin with predominantly follicle-stimulating activity was discovered in the serum of pregnant mares independently by Cole & Hart (1930) and by Zondek (1930). The preparations show luteinizing acitivity, and in 1940 Cole, Pencharz & Goss showed that both activities probably reside in a single substance. Various attempts were made to isolate the hormone, but the most potent preparation obtained up to the present time is that of Rimington & Rowlands (1944), which assayed 12,500 i.u./mg.

i. Sources and extraction

The only available source of PMS is the serum of pregnant mares. The concentration of the hormone in blood reaches a maximum between the 50th and 80th days of pregnancy (Cole & Hart, 1930).

Various methods have been employed to separate PMS from the serum proteins. The best method is undoubtedly that of Rimington & Rowlands (1941); it involves precipitation of inert proteins with metaphosphoric acid at pH 3.6. The recovery of activity in the supernatant fluid is quantitative.

ii. Purification

Rimington & Rowlands (1941) separated the hormone from metaphosphoric acid extracts by adsorption on benzoic acid at pH 4.6, followed by removal of the benzoic acid with acetone. The material was purified further by fractional precipitation from aqueous ethanol at various pH values. The final active fraction was precipitated from 66% aqueous ethanol at pH 1.5 at low temperatures.

iii. Evidence of homogeneity

Rimington & Rowlands (1944) were unable to characterize their material physico-chemically owing to insufficient material.

iv. Chemical and physico-chemical properties

Rimington & Rowlands (1944) reported that their preparation contained 16.9% of hexose and 8.4% of hexosamine. PMS, like CG, therefore has a hexose: hexosamine ratio of 2:1. The biological activity of impure PMS preparations is destroyed by nitrous acid and formaldehyde. It is also destroyed by cysteine and cyanide, presumably by rupture of disulphide bonds. The hormone is inactivated by crystalline pepsin, trypsin, and chymotrypsin and by carbohydrases such as ptyalin, emulsin and takadiastase (Evans & Hauschildt, 1942). These authors have concluded that both the protein and carbohydrate portions of the PMS molecule must be intact for biological activity. Such results must be interpreted with caution, since Rimington & Rowlands (1950) have shown that commercial takadiastase contains an enzyme which is very effective in inactivating PMS. This enzyme is not identical with diastase or *N*-acetylglucosaminidase and is not a proteolytic enzyme. Inactivation by this enzyme is accompanied by the liberation of an ultrafiltrable carbohydrate-containing fragment. It is clear that results of chemical significance can be obtained only by the action of highly purified enzymes on highly purified hormones and that further progress must await the isolation of homogeneous PMS.

5. Concluding Remarks

It will be seen from the foregoing review that only two gonadotrophic hormones have been obtained in a satisfactory state of purity, and that even here, in both cases, there is evidence that the biological activity may reside in a small portion of the molecule or may be due to a trace constituent. In comparison with ACTH, the gonadotrophins have received relatively little attention from the chemist in the past decade and it is very desirable that they should be re-examined by more modern methods of fractionation. All the known gonadotrophins are glycoproteins, and the carbohydrate residue appears in most cases to be essential for biological activity. This fact only emphasizes our ignorance of the chemistry of glycoproteins in general and it would appear that advances in our knowledge of the structure and mode of action of the gonadotrophins must await corresponding advances in this field of protein chemistry.

Addendum

Steelman, Lamont & Baltes (1955) have recently published some work which casts considerable doubt on the homogeneity of previous FSH preparations. By pancreatin digestion of purified FSH preparations, followed by acid and alkaline ethanol fractionation, they have prepared material up to ten times as active biologically as previous preparations. Electrophoretic examination of such material revealed the presence of five components. This work is in general agreement with the findings of Li (1950), and if confirmed will necessitate a complete re-examination of the chemistry of FSH.

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ENDOCRINOLOGY OF THE TESTIS

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1 Effects of castration
2 Transplantation of the testis
3 Origin and identity of the testicular hormones
4 Biology of the androgens
References

Our basic knowledge of the testis as an organ of internal secretion was obtained in classical fashion by studying the effects of removal and transplantation, and by the preparation of active extracts. This sequence of observations extended over a long period, because the effects of castration have aroused scientific and other curiosity since ancient times, while extracts of proved and reproducible androgenic activity were not prepared until the late 1920's. Altogether, a wealth of information has now accumulated, and it has become evident that the testis, by virtue of its secretion of androgen, influences profoundly not only the male reproductive organs and characters, but also many functions of the body not directly connected with reproduction. Thus, castration, among other general effects, has been found in various species to modify body growth and configuration, to affect systemic organs and to have striking metabolic effects. These changes are prevented, or reversed, so far as may still be possible, by the administration of androgens which have, for instance, renotrophic, protein anabolic, and many other effects not directly concerned with the reproductive processes.

In accordance with the general title of this number of the Bulletin, the discussion which follows deals with the endocrinology of the testis only as it relates to the reproductive processes, and it is further limited to androgenic effects in mammals and birds. The organs and characters involved comprise: (i) The secondary sexual characters essentially associated with sex and dependent on the gonads, but concerned only indirectly with reproduction. Such characters are well developed in birds, in which they have proved excellent hormone indicators, but ill developed in laboratory mammals. (ii) The accessory reproductive organs and glands, well developed in mammals, but ill developed in birds.

1. Effects of Castration

Comprehensively it may be said that castration of the immature bird or mammal arrests development of the accessory organs and dependent secondary sexual characters, while castration of the adult causes them to regress. Thus in birds the comb and wattles of the capon are atrophic, or hypotrophic, and crowing is absent; by contrast, the display plumage and spurs of domestic fowl depend not on the presence of testes, but on the absence of an ovary, and are not affected by castration. The meagre accessory organs of male domestic fowl offer little scope for experiment, but the atrophic state of the vasa deferentia in the capon has been recorded and illustrated (Callow & Parkes, 1935).

In mammals, various characters, e.g. the presence of horns, the distribution or nature of hair, the pitch of the voice, and the possession of a characteristic odour in certain species, are sexual in the sense that they are dependent on the presence of a functional testis. In deer, for instance, early castration causes non-development or shedding of the horns (Fowler, 1894), while in the baboon the cape of long grey hair which distinguishes the male from the female disappears at the first moult after castration (Zuckerman & Parkes, 1938). In

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other mammals, some of which were recorded by Darwin, castration abolishes a characteristic male odour by depressing the odoriferous glands, as in the case of the pungent smelling billy goat (Champy, 1931). In man, it is well known that early castration prevents development of the male pattern of pubic and facial hair, and the breaking of the voice at puberty. The results of castration at a later stage are less definite, but may be interesting, as shown by the striking and informative title of Hamilton's (1942) paper, " Male hormone stimulation is prerequisite and an incitant in common baldness ". In mammals, too, a vast literature, fully reviewed by Beach (1947), has grown up on another secondary sexual character—sexual drive. Very early castration may prevent the development of sexual behaviour, but this, like the inhibiting effect of post-pubertal castration, is more evident in laboratory rodents than in primates. The accessory organs of mammals, the development and maintenance of which is dependent on the presence of the testis, include the epididymis, the vas deferens, the penis, the seminal vesicles and the prostate, Cowper's and preputial glands and the scrotum. Earlier observations related mainly to morphological aspects of the organs, their size and the characteristics of their epithelial lining. Later, more attention was devoted to functional activity, and investigations were made into the effect of castration on the capacity of the isolated epididymis to store spermatozoa in a viable state (Benoit, 1925), on the spontaneous contractility and pharmacological responsiveness of the male reproductive tract (Martins & Valle, 1939), on the capacity for erection and ejaculation, and on the volume and coagulability of the accessory secretions (Moore & Gallagher, 1930; Wallace, 1949), on the capacity of the scrotum to relax and contract in response to the stimulus of heat and cold, and thus to perform its thermo-regulatory function (Andrews, 1940), and most recently of all, on the metabolism of citric acid and fructose by the accessory organs (Mann & Parsons, 1947; Mann & Lutwak-Mann, 1951). All these functional activities of the accessory organs are depressed or abolished by castration.

One important rider must be added to this account of the effects of castration on the accessory organs—that small amounts of androgen are produced by the adrenal cortex. This matter is dealt with elsewhere[1] in this number. All that need be said here is that castration of the new-born rat does not immediately stop development of the prostate gland which may show growth and differentiation for several weeks (Price, 1936). Adrenalectomy and castration of young rats, however, stops development of the gland immediately, and an andromimetic effect of the adrenals in the young rat may reasonably be inferred (Burrill & Greene, 1939). No such effect is usually discernible in adult castrated animals, though it has been reported that adrenal adenomata in guinea-pigs castrated several years previously may be associated with redevelopment of the accessory reproductive organs (Spiegel, 1939; Woolley, 1950).

2. Transplantation of the Testis

The observations summarized above make it evident that the testes have a profound effect on many bodily functions. The next question concerned the mechanism involved, and the obvious experiment was to transfer the testis to a new site in which it would have vascular but not nervous connexions. Such a transplantation, carried out by Berthold (1849), seems to have been the first recorded intentional experiment in endocrinology. Berthold noted that cockerels in which the testes had been transplanted retained sexual behaviour and male appearance, and concluded that the effect of the testis was exerted through the blood stream. Since then very numerous researches have demonstrated clearly that testicular tissue is able to carry out its regulatory functions from almost any part of the body, and that the mechanism is therefore humoral rather than nervous in nature. Testicular tissue grafts readily, especially if small pieces of infantile testis are used as autografts. Homografts between different individuals of the same strain often take and survive, and of course function endocrinologically (see, for example, Parkes & Smith, 1954), provided that the host is castrated to ensure that an adequate supply of pituitary gonadotrophin is available to stimulate the graft. Heterotransplants of testis between individuals of different species do not develop or function, though they may remain as histological mummies for a time (see, for example, Browman, 1937). Subcutaneous or intraperitoneal grafts do not normally show full spermatogenesis, owing to the adverse thermal environment, but grafts into the scrotum frequently do so (Moore, 1926) even when developing from tissue preserved in glycerol–saline at the temperature of dry ice (—79° C.) (Deanesly, 1954). Effective transplantations of testis tissue can be made into the eye (Turner, 1938) where the graft is less subject to immunological stresses and its progress can be watched. Transplantation direct into the target organ may provide an intense local stimulation (Jost, 1948). By contrast, intramesenteric transplantation results in the androgen being secreted into the portal system and partially or wholly destroyed in the liver before reaching the target organ (Burrill & Greene, 1940). A particularly effective demonstration of endocrine activity is given by a testicular graft into a spayed female, in which the heterosexual rudiments, notably the clitoris, are stimulated by androgen from the graft (Steinach, 1913; Lipschütz, 1917).

3. Origin and Identity of the Testicular Hormones

The evidence summarized above, together with that discussed in section 4 below, shows clearly that the testis secretes into the blood stream one or more substances which evoke the attributes of maleness, notably by promoting the development of the secondary sexual characters and accessory organs. Such substances can properly be called androgens, and the chief one, perhaps the only one, elaborated and secreted by the testis is testosterone, originally isolated from testicular extracts in 1935 (David, Dingemanse, Freud & Laqueur, 1935). The chemistry of these substances is dealt with in the paper by R. K. Callow.[2]

Two problems are relevant here: what part of the testis is responsible for the elaboration of androgen, and does androgen constitute the only type of internal secretion produced by the testis in the performance of its endocrine functions?

The testis consists essentially of seminiferous tubules embedded in interstitial material composed of connective tissue, blood vessels and small clumps of cells of secretory type—the so-called interstitial or Leydig cells. In the early years of this century, the question of whether the internal secretion of the testis originated in the tubules or the interstitial tissue became a major biological controversy (see Lipschütz, 1924). Fortunately, several different ways were found of causing severe damage to the tubules without

[1] See Chester Jones, p. 156 of this number of the Bulletin.—Ed.

[2] See Callow, p. 126 of this number of the Bulletin.—Ed.

injuring the interstitial cells. Thus, artificial cryptorchidy (Moore, 1922), grafting to abnormal sites (Steinach, 1910), *x* irradiation (Bergonié & Tribondeau, 1904), and, for a time, ligation of the efferent ducts (van Wagenen, 1924) all cause damage to the tubules preferentially. In extreme cases the contents of the tubules are reduced to Sertoli cells only, and sterility is irreversible. Decreasing damage leaves increasingly more cells of the seminal line, and fertility may be recovered if the ducts are intact.

Endocrinologically, the end result of the various treatments is probably the same, though the mechanism may be quite different. Artificial cryptorchidy or transplantation of testicular tissue to intraperitoneal or subcutaneous sites exposes the tubules to a temperature several degrees higher than that normally found in the scrotum and, for whatever reason, such a temperature is incompatible with the continuance of spermatogenesis in the testes of most mammals, as can further be shown by the application of heat or even merely of thermal insulation to the scrotum (Moore, 1924; Phillips & McKenzie, 1934). In birds and certain mammals, notably the marine ones, the testes are normally abdominal, so that there is no inherent incompatibility between spermatogenesis and normal body temperature in homeothermal vertebrates. In these circumstances the development of the hypothermal scrotum—at best an inconvenient organ—and the associated adaptation of spermatogenesis to a temperature below that of the body as a whole must be regarded as an evolutionary oddity.

The sensitivity of the seminiferous tubules to irradiation damage seems to depend on the general principle that the most actively dividing cells are the most sensitive to ionizing radiations. *x* Irradiation or other irradiation has therefore provided a most useful tool for several kinds of research on the reproductive processes, but does not call for further comment here.

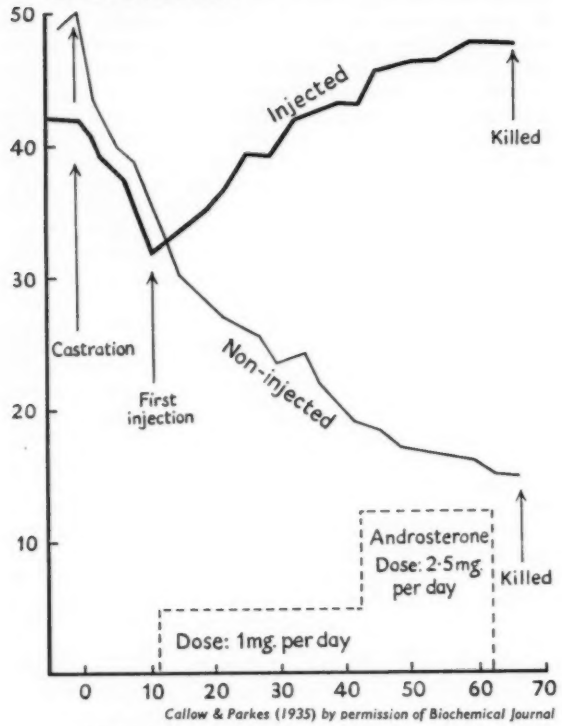
The effects of ligation of the spermatic ducts are of more general interest. It was claimed long ago (Bouin & Ancel, 1903) that ligation of the vas deferens led not only to degeneration of the spermatogenic tissue of the tubules but to hypertrophy of the interstitial cells. This claim was enthusiastically endorsed by Steinach (1920), who further believed that the operation had a rejuvenating effect not only on old animals but also on old men. Steinach's postulation involved three assumptions, for none of which was there adequate evidence, viz. that vasoligation caused degeneration of the tubules, and stimulated the interstitial cells to produce more androgen, the lack of which was the primary cause of senescence. Later work, especially by Moore and his colleagues (for example, Moore & Oslund, 1924), showed conclusively that vasectomy produced no regular effects either on tubules or interstitial tissue, and destroyed the rickety foundations of Steinach's original concept. The eclipse of vasectomy was followed by experiments on ligation of the efferent ducts between the testis and the epididymis. This procedure obviously builds up back-pressure in the testis to a greater extent than does ligation of the vas deferens, which leaves the epididymis to absorb the products of spermatogenesis, and at least temporary degeneration of a large proportion of the tubules may be obtained by this method (see Harrison, 1953, for review). This operation was popularly known at one time as Steinach II, but seems to have been equally as ineffective as vasectomy in causing rejuvenation.

All this work has led to one general endocrinological conclusion—that destruction of the contents of the seminiferous tubules, leaving the interstitial tissue intact, does not interfere with the capacity of the testis to promote and maintain the usual attributes of maleness. It is reasonable to deduce therefore that the testicular androgen originates outside the tubules, presumably in the interstitial cells. This conclusion, however, relates only to androgens and requires amplification in two directions. First, there is some evidence that the testis produces a second, non-androgenic, but essential internal secretion associated with the tubules rather than with the interstitial tissue. This idea arose in the first place from the observation that castration changes appeared in the anterior pituitary gland of the rat after severe damage to the tubules by radium irradiation, in spite of the fact that the interstitial cells had remained intact (Mottram & Cramer, 1923). Similarly, doses of testosterone adequate to restore the accessory organs in the castrated rat do not reverse the castration changes in the pituitary gland (Törnblom, 1942). Development of these observations led to the idea that the tubules produce a separate hormone concerned mainly in regulating the supply of follicle-stimulating hormone (FSH) from the pituitary gland. Second, it must be remembered that the testis is known to elaborate and probably secrete oestrogen, as shown by the isolation of oestrone and oestradiol from extracts of horse testis (Beall, 1940), and by the fact that the stallion, but not the gelding, excretes fabulous amounts of oestrogen in the urine (Zondek, 1934). There is also evidence to associate oestrogen production by the testis with the tubules rather than with the interstitial tissue (Huggins & Moulder, 1945). It is possible, therefore, but far from certain, that the testicular oestrogen is the agent responsible for inhibiting the secretion of pituitary FSH. There is, however, some direct evidence of yet a third internal secretion of the testis. Thus, McCullagh (1932) found that the administration of aqueous extracts of testis led to the disappearance of castration changes in the anterior pituitary gland without stimulation of the accessory reproductive organs, and he postulated the existence of a water-soluble pituitary-inhibiting hormone to which he gave the name "inhibin". Other authors have inferred the production of an X hormone from the Sertoli cells, having a function similar to that of the hypothetical inhibin, but not necessarily being water-soluble (Howard, Sniffen, Simmons & Albright, 1950). This whole matter has recently been reviewed (Bishop, 1954) but the simple fact remains that, in spite of much work, there has not yet been isolated from the testis any non-androgenic, non-oestrogenic substance with pituitary-depressing properties.

4. Biology of the Androgens

The fact that the androgen-secreting tissue of the testis constitutes a very small proportion of the whole organ naturally complicates the preparation of active extracts. Nevertheless, Moore and his colleagues in 1927–29 provided conclusive evidence of having obtained extracts capable of stimulating the secondary sexual characters and accessory reproductive organs in castrated birds and mammals. This early work was fully reviewed by Moore, Gallagher & Koch (1929) and Koch (1937). Little more progress was made for a time owing to the difficulty of producing adequate quantities of purified extracts. In the early 1930's, however, the isolation of androsterone from urine by Butenandt and of testosterone from testis extracts by Laqueur and his colleagues,

FIG. 1. MAINTENANCE OF COMB SIZE BY INJECTION OF ANDROSTERONE AFTER CASTRATION OF ADULT COCKEREL



Callow & Parkes (1935) by permission of Biochemical Journal

Abscissae: time (days)
Ordinates: size of comb (length × height) (cm.²)

and the preparation of these substances and a whole series of other androgens from cholesterol by Ruzicka as described in the paper by R. K. Callow[3], changed the situation dramatically, and within a few years abundant material became available for biological work. As a result the earlier studies on birds were quickly confirmed and made quantitative (fig. 1), and the work on mammals was amplified by the complete reversal of castration effects in laboratory rodents and extended by investigations on other species, including man and monkeys.

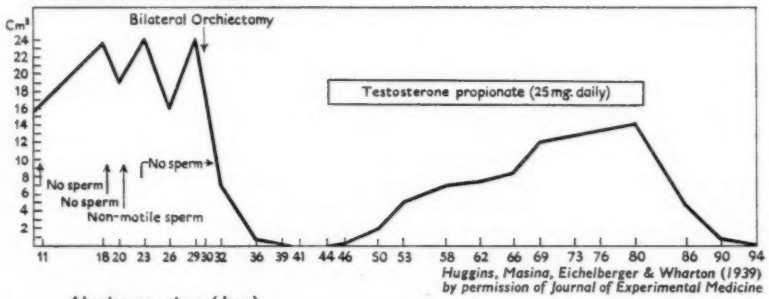
It is now possible to say that all the recognized secondary sexual characters and accessory reproductive organs in species so far examined can be restored or maintained after castration, or can be developed prematurely in the immature animal, by the administration of adequate amounts of testosterone. In mammals, such secondary sexual characters as the scrotal pigmentation of the ground-squirrel (Finkel, 1945), the rainbow hues of the male drill, and the long pectoral hair of the baboon (Zuckerman & Parkes, 1939), and male-type pubic and facial hair in man (Kenyon, 1938), can all be evoked by administration of testosterone. Sexual behaviour in the castrated mammal is also restored, its various components returning on continued treatment with testosterone in the reverse order to that in which they disappeared after castration (Beach, 1947). As regards the restoration of the accessory reproductive organs in the male, detailed references need not be given to the mass of work on the restoration of gross size of the prostate, seminal vesicles, and other accessory glands and organs in laboratory rodents. Recent work, as already stated, has dealt mainly with functional aspects, and results relating to the volume of prostatic secretion are shown in fig. 2, and to the content of fructose in the seminal vesicles in fig. 3.

This summary of the effect of testosterone on the secondary sexual characters and accessory reproductive organs is highly elliptical, but it indicates that the effects produced by the hormone are extremely diverse. At the tissue level they involve hyperaemia, oedema, cell proliferation and secretory and metabolic changes, but, clearly, a great deal remains to be elucidated about the finer mechanisms.

The lesser androgens produce the same effects qualitatively as testosterone, though in the case of the very weak ones, e.g. dehydro*epi*androsterone, massive dosage over long periods may be required to obtain substantial effects with the more refractory indicators. At an early stage in the work with crystalline androgens it was commonly supposed that the relative potency of different compounds might be different on different organs. It is evident, however, that very large-scale quantitative research would be required to establish such a thesis, because threshold levels are different for different organs, and the conditions for optimal effectiveness vary greatly with different compounds. For these reasons, also, the relative activities of the various androgens can be determined only in a general way, as is clearly shown by the results of Tschopp (1936), Deanesly & Parkes (1936a), Korenchevsky & Dennison (1936) and Kochakian (1944, 1952).

The problem of effectiveness very soon became of great importance in the study of the crystalline androgens, as it had previously become in the study of the purified or crystalline oestrogens. The basic fact is that the testis normally performs its androgenic functions by steady regular secretion of androgen at an appropriate level. It follows, therefore, that to produce efficiently the effects of endogenous androgen by

FIG. 2. EFFECT OF CASTRATION, AND OF STARTING AND STOPPING TESTOSTERONE INJECTION, ON VOLUME OF PROSTATIC SECRETION OF THE DOG



Huggins, Masina, Eichelberger & Wharton (1939) by permission of Journal of Experimental Medicine

Abscissae: time (days)

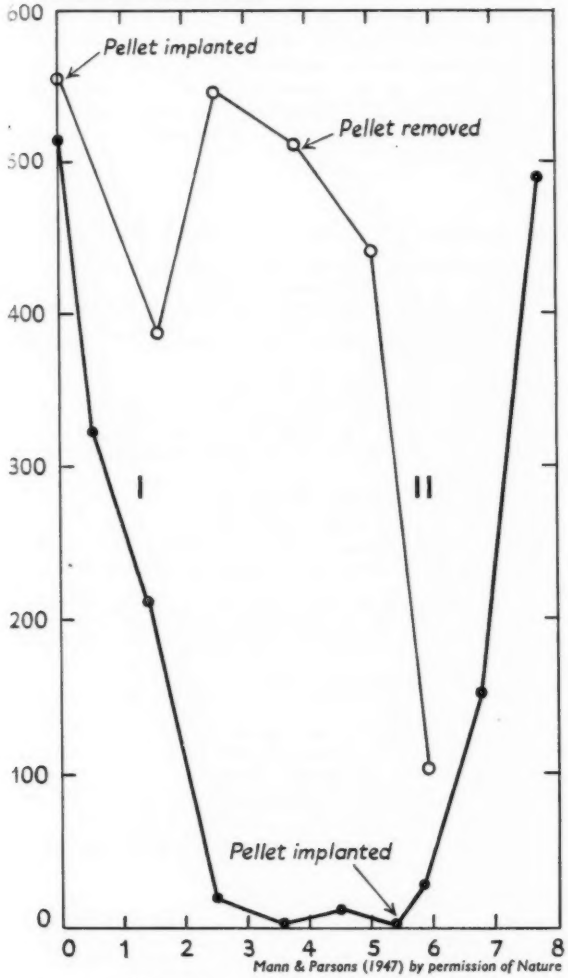
Ordinates: prostatic secretion (ml.) collected during one hour following an intravenous injection of pilocarpine (6 mg.)

[3] See Callow, p. 126 of this number of the Bulletin. —Ed.

the administration of exogenous substances requires that the method of administration shall be such as to provide a steady threshold supply to the responding organ. An injection once daily of a substance which is absorbed and used or destroyed in a few hours, for instance, produces an inefficient alternation of excess and deficiency. In such cases effectiveness can be greatly increased by increasing the number of injections into which the total dose is subdivided, or by any method which decreases the rate of absorption from the site of injection (see fig. 4). This simple fact has been responsible for a great deal of the work as well as for many of the misconceptions relating to the androgens.

In the course of isolating testosterone from testis extracts, Laqueur and his colleagues found with some surprise that during final purification the preparations appeared to lose activity, which could be restored by the recombination of discarded inert fractions (Freud, Dingemanse & Polak, 1935). Soon afterwards it was shown by Miescher and his colleagues (Miescher, Wettstein & Tschopp, 1936) that the same result could be obtained by the addition of a variety of substances, notably fatty acids. This enhancement, however, was seen only if the fatty acid was mixed with the solution of testosterone; simultaneous administration to a different part of the body was ineffective. It was obvious, therefore, that absorption from the site of injection was made more efficient, presumably by being delayed, by the presence of the insoluble material (Deanesly & Parkes, 1936b). A further decisive step forward was taken by Miescher and his colleagues (Miescher *et al.* 1936) in the preparation of a long series of aliphatic esters of testosterone, with solubility in body fluids depending on the length of the acid chain. Several of these esters were far more effective than testosterone by ordinary methods of injection, and the propionate was selected as being the most suitable for general use, and has since become the most widely used form of the hormone. The esters show no prolongation of effect when given intravenously, so that the effect of esterification is clearly to delay absorption from the site of injection.

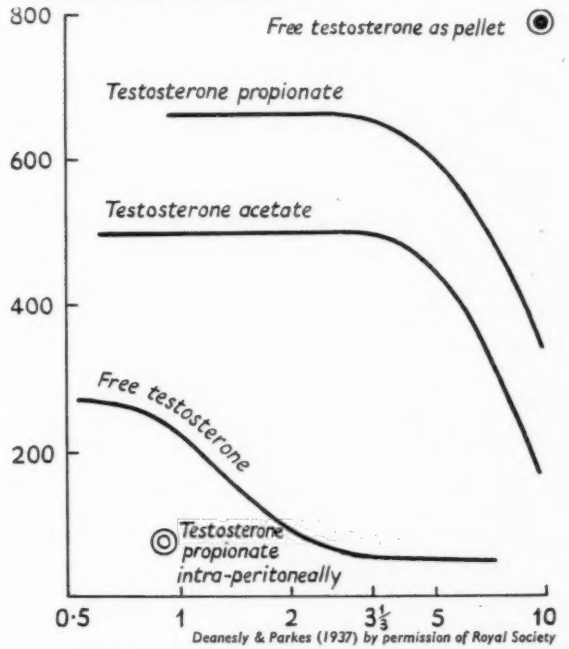
FIG. 3. EFFECT OF CASTRATION, AND OF IMPLANTATION AND REMOVAL OF 100 mg. PELLET OF TESTOSTERONE, ON FRUCTOSE IN THE EJACULATE OF THE RABBIT



Mann & Parsons (1947) by permission of Nature

Abscissae: weeks after castration
Ordinates: fructose per ejaculate (μg.)

FIG. 4. WEIGHT OF THE PROSTATE AND SEMINAL VESICLES IN THE CASTRATED RAT TREATED WITH TESTOSTERONE



Deanesly & Parkes (1937) by permission of Royal Society

Abscissae: interval between injections (days) (logarithmic scale)
Ordinates: total weight of prostate and seminal vesicles (mg.)
Curves: testosterone administered subcutaneously in oil solution
Figure shows effect of number of injections (total dose approximately 2 mg. in all experiments), given during 10 days, according to the form of the hormone and the method of administration.

Another method of securing slow and effective absorption was evolved by Deanesly & Parkes (1937), who observed that androstanediol was very effective when injected as a mush of crystals, and were, therefore, led to try the effect of implanting large crystals, or compressed pellets, of testosterone and other androgens. These experiments were highly successful and laid the foundations of the tablet implantation technique.

A major factor influencing the effectiveness of an administered hormone is, of course, the route of administration. The comments made above refer mainly to subcutaneous or intramuscular administration, but several of the considerations apply to other routes of administration. Thus, the brief intense stimulation given by intravenous injection is highly ineffective in the case of the androgens, and intraperitoneal injection may be very little better. By contrast, inunction of the hormone in an appropriate medium to the skin may be quite effective, especially where prolonged low dosage is required, while local administration to the target organ may, of course, produce results with a dosage far less than that required by any other method (Fussganger, 1934). In oral administration yet another consideration appears, i.e., the substance must have at least a chance of reaching the target organ before being metabolized. The comparative inactivity of most of the androgens by mouth appears to be caused not by lack of absorption but by destruction of the substance in the liver before it enters the systemic circulation (Dorfman & Hamilton, 1939). Probably, therefore, the 17-methyl derivative of testosterone, which has substantial activity by mouth, is protected to some extent against being rapidly metabolized.

Most of the properties of the androgens which can reasonably be called androgenic are referred to above. In addition, several of this group of substances have striking ambisexual activity in the sense that they cause enlargement of the reproductive tract in the ovariectomized female (Korenchevsky & Hall, 1937). Moreover, some of the methylated derivatives, and to a lesser extent testosterone, have a definite progesterone-like action in the usual test on the uterus of the rabbit (Klein & Parkes, 1937). In the same way many of the androgens resemble progesterone in that they inhibit some of the effects of oestrogen, notably cornification (Korenchevsky & Hall, 1937) and fibromatogenesis (Lipschutz, 1950). Finally, some pituitary-inhibiting property seems to be demonstrated by the retardation of testis growth in young rats chronically treated with testosterone (Moore & Price, 1937), but, as mentioned above, it is not certain if an androgen-pituitary mechanism plays any part in normal events. Nor is it possible to interpret the observation that spermatogenesis can be maintained for a time in rats after hypophysectomy by the administration of androgens (Walsh, Cuyler & McCullagh, 1934), though the maintenance of the scrotal position of the testis by the treatment is no doubt a contributory factor.

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BIOLOGY OF THE OESTROGENS

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1 Embryonic differentiation
2 Effect of oestrogen in the adult female
3 Effect of oestrogen in the male
4 Histogenesis of oestrogen-sensitive tissues
5 Oestrogens and cancer
6 Effects of oestrogen at the cellular level
7 Mechanism of the menstrual cycle
8 Effects of oestrogen on behaviour
9 Control of oestrogen activity
References

Our knowledge of oestrogens stems from experiments whose aim was to reverse the involution that occurs in the uterus and vagina after bilateral ovariectomy (cf. Marshall & Jolly, 1906). The identification of the active principle in the extracts that were used in these studies, which date from the turn of the century, had, however, to wait until Allen & Doisy (1923) hit on the idea of treating vaginal cornification as a specific index of oestrogenic activity. The ease of this test, which derives from Stockard & Papanicolaou's (1917) discovery of the vaginal cycle in the guinea-pig, and the opportunity which it afforded different laboratories for standardizing their observations, immediately stimulated an immense amount of research, and it was not long before " pure " oestrogenic hormone was isolated from ovarian tissue, human placenta and the urine of pregnant women. In 1929 and 1930 the first crystalline oestrogens were almost simultaneously and independently obtained by Doisy and his collaborators in the United States of America; by Butenandt in Germany; and by Marrian in Great Britain; and it was quickly recognized that all the oestrogenic substances which could be isolated from body tissues and fluids were closely related steroids. This early work, of which a full share was borne by scientists in Great Britain and the Commonwealth, is summarized by Parkes (1929) and by Allen (1939). It led to a clear appreciation that oestrogens not only control the accessory reproductive organs, but that they also have a general influence on developmental and metabolic processes, as well as on the total behaviour of the individual. It is not possible, in a brief review, to deal with more than a few of the themes which interrelate the major biological effects of the oestrogens. The first that needs to be considered is the nature of the tissues that are sensitive to oestrogens, and the part played by oestrogens in development.

1. Embryonic Differentiation

Whatever its genetic sex, every embryo begins with the same basic embryonic reproductive tissues, consisting of a pair of Müllerian ducts, which are the first manifestation of the female reproductive tract, and a pair of Wolffian ducts, from which the male accessory reproductive organs develop. Both sets of ducts are attached to a urogenital sinus which, in the earlier stages of development, is identical in the two sexes. This initial phase is a sexually indifferent stage—by which is usually meant a phase of development that is not under hormonal control.

The ambisexuality of the organism is manifested in the fact that these basic embryonic reproductive tissues will evolve either in a male or female direction, depending on the nature of a " chemical " stimulus which they receive in the next stage of their development. This is determined by the interaction of an undefined number of mutually-opposing male and female genes carried on the autosomes and the sex chromosomes. If the foetal gonad becomes a testis it produces " male hormone ", and this stimulates the development of the Wolffian duct and suppresses that of the Müllerian. It also induces a masculine differentiation of the urogenital sinus and of the primordial external genitalia. The opposite happens if the gonad develops into an ovary.

Whether or not the inducing substances are chemically the same as the sex hormones that are produced by the mature gonads is not known. Experiment has, however, shown that " adult " male sex hormone can reverse the normal course of organogenesis in a genetically female foetus, provided it acts at an early enough stage. The part played by oestrogenic hormone is much less clear. One view is that it is directly responsible for the growth and differentiation of the Müllerian ducts. Another is that the latter differentiate autonomously, given only that the organism is not under the influence of some male inducing or androgenic substance. This conclusion emerges from a variety of observations, of which the significant one is that the Müllerian ducts will not only start differentiating into the female accessory reproductive organs after removal of the foetal ovaries, but that they will also do so in the male foetus if the testes are removed early enough (Jost, 1950, 1955: experiments on rabbits). On the other hand, it is equally well established that oestrogens can reverse the trend of development of the gonads and accessory organs in a genetic male opossum (Burns, 1950), and also that the uterus and vagina do not develop fully in rats and rabbits except when stimulated by an oestrogen (cf. Raynaud, 1950; Jost, 1950). Moreover, oestrogen, through its action on the urogenital sinus, will cause major feminine transformations in the distal part of the reproductive tract in the male foetus of all species that have been tested. For these reasons, it is reasonable to suppose that oestrogen, or some analogous ovarian secretion, plays a part in the natural process of development of the definitive reproductive organs of the female (Zuckerman, 1950). It is worth noting that in a few species, including man, the body fluids of the maternal organism contain a very high concentration of oestrogen in the latter part of gestation. This oestrogen is able to pass through the placenta, and male and female babies are usually born showing some signs of oestrogenic stimulation of the uterus, the uterus masculinus, and the mammary duct system (Courrier, 1924, 1928; Sharpey-Schafer & Zuckerman, 1941).

2. Effect of Oestrogen in the Adult Female

In the adult organism oestrogens (i) control certain general physiological changes, such as alterations in water metabolism; (ii) effect changes in the state and function of various endocrine organs, including the anterior pituitary and adrenal

cortex; and (iii) stimulate growth of the epithelial and fibro-muscular tissues of the male and female urogenital tracts, and also of certain non-reproductive tissues, such as skin and gums. A review of these various effects has to begin with a consideration of the changes that take place in the female reproductive organs themselves.

Reproductive capacity in the non-pregnant female mammal manifests itself by the cyclical recurrence of structural changes in the reproductive organs, and in the cyclical repetition of periods of heat or oestrus, during which the animal is receptive to the male. In some animals these oestrous cycles continue uninterruptedly throughout the year; in others they are restricted to breeding-seasons of varying duration. Some species may experience only one, while others undergo many cycles during a season. The duration of the cycle itself varies considerably (e.g. about four days in the rat, 28 days in man). Furthermore, while most species are receptive only about the time of ovulation, some (e.g. man) do not experience restricted phases of oestrus. In all species, however, the cycle ceases after the ovaries are removed. It is, therefore, reasonable to regard the ovary as the essential source of oestrogenic hormone in the adult animal, and the reproductive tract as its focus of action.

The dominant event in the oestrous cycle is ovulation. This occurs spontaneously in most species that have been studied (e.g. man, rat), but in a few it normally occurs only after the stimulus of coitus (e.g. ferret, rabbit). The pre-ovulatory or follicular phase of the cycle is marked by a wave of epithelial proliferation in the vagina and uterus, and the post-ovulatory or progestational phase, during which the ruptured ovarian follicle or follicles are becoming transformed into corpora lutea, by secretory changes in the glands of the uterine mucosa. This change is mainly under the influence of the hormone progesterone, produced by the luteal cells of the ovary.

The accessory reproductive organs become very reduced in size after ovariectomy. Thus, the vaginal epithelium in a spayed rat consists of only two or three layers of low cuboidal cells instead of, as in the normal animal at the height of oestrus, about ten layers of epithelial cells. Oestrogen restores the normal condition. It causes intense mitotic activity in the vaginal mucosa, which in consequence becomes greatly stratified. The superficial cornified layers of cells are shed into the vaginal lumen, and a smear of the contents of the vagina reveals only cornified epithelial cells. As soon as treatment with oestrogen is stopped, the wave of proliferation ceases, and the vaginal wall becomes invaded by leucocytes, which also penetrate to the vaginal lumen, and so appear in the vaginal smear. A vaginal cycle, as revealed by cyclical changes in the cellular nature of the smear, occurs in all normal female mammals, but is not as marked in some (e.g. man, sow) as in others (e.g. rat, guinea-pig). The peak of proliferation and cornification almost always occurs about the time of ovulation.

The basic changes which oestrogen stimulates in the uterus are hyperaemia, oedema, and then cellular proliferation, but the general appearance of the oestrogen-stimulated uterus varies fairly considerably between species, as contrasted with the more uniform appearance of the oestrogen-stimulated vaginal epithelium. In the rat the uterus becomes hyperaemic and thin-walled as a result of distension with fluid. In the rabbit there is relatively less oedema, and in cross-section the uterine lumen remains stellate in shape. In the monkey there is a far greater development of glandular tissue. The motility of the uterine muscle increases when it is under the influence of oestrogen.

Oestrogen also stimulates hyperplasia and hypertrophy of the epithelium and fibro-muscular elements of the uterine tubes, and of the epithelial and connective tissue of the external genitalia, including, in some species, the contiguous skin (see below). Apart from these effects, oestrogen also promotes the growth of the nipples and the duct system of the mammary gland (see Folley, p. 145 of this number of the Bulletin), as well as epithelial growth, with superficial cornification, of the gums (Ziskin, Blackberg & Slanetz, 1936).

Extragenital effects of oestrogens in non-mammalian forms include the control of the plumage of the female bird. According to Parkes & Emmens (1944), in every case in which ovarian hormones determine the feathering of a hen, growing feathers in the cock or capon bird can be changed to the female type by the administration of an adequate dose of oestrogen.

3. Effect of Oestrogen in the Male

The male tissues which are sensitive to oestrogens are mainly those, such as the coagulating glands of the mouse prostate and the uterus masculinus of the primate prostate, which are embryologically derived from either the Müllerian ducts or the neutral tissue of the urogenital sinus. In the mouse the first tissue to respond is a cyst which is occasionally found in the situation where the urethral end of Müller's duct would be expected to occur. Then in order of response come the coagulating glands, the seminal vesicles, the periurethral glands (including Cowper's glands) and the entire urethra, the other lobes of the prostate, the vas deferens and, finally, the epididymis. In all the organs which open into the urethra, the change, which consists of squamous metaplasia of the normal transitional or low cuboidal epithelium, begins in the urethral parts of the collecting ducts and spreads peripherally into the organ (Burrows, 1935). These changes occur in the absence of the pituitary (de Jongh, 1934) and are associated with fibro-muscular growth in the reacting organs (Freud, 1933; Korenchevsky & Dennison, 1934, 1935; Overholser & Nelson, 1935; de Jongh, 1935; Korenchevsky, Dennison & Brovsin, 1936).

Corresponding changes take place in all other animals that have been tested, including man (Sharpey-Schafer & Zuckerman, 1941), but the details differ from species to species. For example, the prostate of the dog is very sensitive to oestrogenic stimulation, and not only the uterus masculinus and collecting ducts, but also the secreting acini, undergo stratified squamous metaplasia, until the glandular system is converted into a series of cysts, each filled with a core of desquamated epithelial cells (de Jongh & Kok, 1935). The entire urethra also undergoes squamous metaplasia, the upper prostatic urethra apparently being as sensitive as the rest of the tract. These prostatic effects are identical with those of a pathological condition which occurs spontaneously in the dog (Zuckerman & Groome, 1937; Zuckerman & McKeown, 1938; Huggins & Clark, 1940), and originally were believed to bear some relation to the genesis of benign prostatic hypertrophy in man.

4. Histogenesis of Oestrogen-Sensitive Tissues

Prolonged and excessive stimulation by oestrogen may lead in the female either to cystic glandular hyperplasia of the endometrium (with irregular penetration of the hypertrophying

myometrium by the hyperplastic uterine glands—e.g. in man, monkey), or to a process of " metaplasia " in which the normal secretory cylindrical epithelium of the cavum uteri and glands becomes transformed into stratified squamous epithelium. The latter change occurs throughout the uterus in rats (e.g. Selye, Thomson & Collip, 1935); mice (Gardner, Allen & Strong, 1936); and rabbits (Pierson, 1938), but only in the cervix of guinea-pigs and rhesus monkeys, close to the line of junction of vaginal and uterine epithelium (Overholser & Allen, 1933; Engle & Smith, 1935; Lipschutz, 1937; Zuckerman, 1937a). This region in the rhesus monkey is normally an unstable zone of transition between cylindrical and stratified squamous epithelium, and it contains foci of " metaplasia " even in untreated animals (Sandys & Zuckerman, 1938).

These and related observations make it clear that, under normal physiological conditions, oestrogenic sensitivity manifests itself histologically both in the orderly proliferation of secretory cells, and in the proliferation, cornification and desquamation of stratified squamous epithelium. Both types of response also appear under abnormal conditions of stimulation—for example, during prolonged and intense treatment with oestrogenic hormone—but, whereas each is characteristic of definite tissues under normal conditions, tissues which usually undergo orderly glandular proliferation (e.g. the endometrium of the rat) may become transformed into stratified squamous epithelium under non-physiological conditions. The occurrence of metaplastic changes is not only dependent on the duration and intensity of stimulation, but also, to some extent, on the age of the animals subjected to treatment (de Jongh & van der Woerd, 1939).

In most species that have been tested, the glandular response is mainly confined to tissues (e.g. uterus, hydatids of Morgagni) that are undoubtedly derived from the Müllerian duct. The squamous response, on the other hand, occurs in tissues that are believed to have widely different origins. Closer analysis suggests, however, that stratified squamous proliferation or metaplasia is usually a primary response of tissue in whose development oestrogen-sensitive entodermal sinus epithelium has played a part (Zuckerman, 1936a, 1940). In deriving this working hypothesis, it was argued that epithelium of the urogenital sinus tends to track away from the sinus along the paths formed by structures, such as the Müllerian and Wolffian ducts, which obtain attachment to it. This general view has been substantiated by a number of more recent embryological studies of the development of the urogenital sinus (e.g. in the mole: Godet, 1950).

The responses to oestrogens of tissues in whose formation the urogenital sinus has played a part (for example, the vagina) merge insensibly with those of the external genitalia and circumgenital skin. The hypothesis was accordingly extended, with particular reference to primates, to include the thesis that ectoderm plays a part in the development of the sinus (Zuckerman, 1940). Since then Burns (1942) has shown that the ectodermal part of the primitive cloaca plays a large part in the formation of the urogenital sinus of the opossum, and he suggests that " histological identity and identity of physiological response, on the part of stratified squamous epithelium in all parts of the developing urogenital tract " is indicative of their common origin from the ectoderm. The same conclusion emerges from the observations of Bolliger & Carrodus (1939) and Bolliger & Tow (1946) on the phalanger (*Trichosurus vulpecula*). The scrotum of this animal can be completely transformed into a marsupial pouch if oestrogen is administered subsequent to castration. These Australian workers have thus linked the oestrogenic sensitivity of the mammary apparatus with that of the circumgenital skin, and the development of the mammary line with that of the genital zone.

5. Oestrogens and Cancer

The metaplastic changes which oestrogen stimulates at zones of epithelial transition bear on the general question of their influence on neoplastic growths. Under normal conditions it would seem that stratified epithelium does not continue to invade those reproductive organs whose original epithelium it had not completely replaced by the time of birth, partly because of the lack of a sufficient oestrogenic stimulus, and partly because of the counter-influence of the factors upon which the normal condition of the organs in question depends. The point where glandular and stratified squamous epithelium meet in these organs is an unstable zone of transition—such, for example, as is found in the cervix uteri. When the oestrogenic stimulus becomes dominant, stratified epithelium gradually replaces or erodes the normal glandular mucosa, the change usually beginning in the region closest to the original urogenital sinus. When the stimulus weakens, the stratified epithelium recedes, and the mucosa returns to its normal condition. The metaplastic changes which occur spontaneously in the prostate of the dog appear to be due to the pathological secretion of oestrogen by the cells of Sertoli (Zuckerman & McKeown, 1938; Huggins & Moulder, 1945). There is, however, no reason to suppose that these changes are malignant, in contrast to the condition which exists in certain strains of mice that are particularly sensitive to oestrogen. In general it seems that not enough is yet known to decide the extent to which genetic or hormonal factors are responsible for those malignant changes in which oestrogens appear to be involved (cf. Burrows & Horning, 1952).

6. Effects of Oestrogen at the Cellular Level

It is tempting to seek a unitary explanation for the cellular growth that oestrogens stimulate in so many parts of the body. Unfortunately none is yet available. The idea that oestrogens are general mitogenic hormones, affecting practically all tissues (Bullough, 1946) is not borne out by more recent and critical study (Carter, 1953). Our knowledge of the steps of the protein synthesis that oestrogen stimulates in certain tissues is also very limited. We know that alkaline phosphatase and nucleic acids increase in the uterine mucosa as it proliferates, and also that oestrogen will not exercise its full protein anabolic effects in the absence of folic acid, which is concerned in nucleic acid synthesis (Jeener, 1948; Silver, 1954). It is also known that cortisone and compound F will inhibit the influence that oestrogen has on the metabolic processes of oestrogen-sensitive tissues (Szego & Roberts, 1953). On the other hand, Silver (1954) found that the endometrial oedema that is stimulated by oestrogen is not impaired by folic acid deficiency. These and other observations (cf. Lieberman & Teich, 1953) clearly do not add up to a precise picture of the way oestrogen acts at the cellular level. One effect—that on water metabolism—can, however, be usefully considered further.

It has already been noted that one of the three basic changes

that oestrogen induces in the uterus is oedema (the other two being hyperaemia and cellular growth). This effect is part of a generalized pattern of change in water content which the hormone stimulates in most tissues of the body (Zuckerman, Palmer & Hanson, 1950). In monkeys and apes oedema is most conspicuous in the circumgenital or sexual skin, which extends dorsally around the base of the tail, and ventrally over the pubic region. In the mature rhesus monkey this area of skin reddens during the course of the menstrual cycle, the peak of the reaction occurring about the middle of the cycle. In the baboon and pig-tailed macaque, the predominant change is swelling, which reaches a maximum just before the midpoint of the cycle, when ovulation occurs.

The sexual skin response, which undergoes a long process of maturation before it appears as the adult type of reaction (Zuckerman, van Wagenen & Gardner, 1938), is due to hyperaemia (Collings, 1926; Aykroyd & Zuckerman, 1938), and the retention of water (Krohn & Zuckerman, 1937). The total amount of fluid that may be retained is considerable, and may amount to more than 20% of the normal body-weight. When the skin suddenly subsides at ovulation, the volume of urine that is excreted increases considerably, and there is also a rapid fall in body-weight. These events are directly correlated with changes in the daily water balance.

Sexual skin which has been denervated will respond to oestrogen (Zuckerman, 1936b), as will also skin that has been transplanted from its normal site to the chest wall (Bachman, Collip & Selye, 1936; Zuckerman *et al.* 1938). Furthermore, it has been found that neither progesterone nor testosterone nor adrenal cortical hormone is able to retain in the sexual skin water that has been deposited there under the influence of oestrogenic stimulation (Zuckerman, 1939). These observations suggest that oestrogen induces some reversible chemical change either on the capillary wall, the lymphatics, or the extra-capillary tissue, which determines the retention or otherwise of extracellular water.

The swelling is associated with considerable growth of the connective tissue cells, and also with the discharge into the intercellular spaces of protein and presumed mucoprotein (Aykroyd & Zuckerman, 1938; Ogston, Philpot & Zuckerman, 1939). The viscosity of the exudate that drips out of the swollen skin when it is incised is rapidly reduced when the fluid is incubated with testicular hyaluronidase (Chain & Duthie, 1940). This observation suggested that the mucoid which Ogston *et al.* (1939) identified by means of ultracentrifuge studies was a salt-like combination of hyaluronic acid with protein. Chain and Duthie's observation has been confirmed in vivo by Duran-Reynals, Bunting & van Wagenen (1950) and by Krohn (unpublished observations, 1951), who showed that the swelling could be rapidly dissipated by injecting into it hyaluronidase. Duran-Reynals and his colleagues also concluded that the mucoid of the exudate consists largely of hyaluronic acid, which can form highly hydrated gels, and chondroitin sulphate. These two acid mucopolysaccharides are held in different ways in various tissues.

The presence of chondroitin sulphate in the exudate was not confirmed by Rienits (1953), using paper electrophoretic techniques which were delicate enough to disclose the presence of other mucopolysaccharides and that of serum albumin. Rienits also found that there is a direct relation between the hyaluronic acid concentration of the sexual skin and its water content. He found, too, that the hyaluronic acid is present both in the free fluid within the sexual skin (" the sexual skin exudate ") and in a presumably insoluble form bound to the tissues. The concentration of exudate hyaluronic acid is least when the sexual skin is fully swollen, and increases as the skin collapses, the reverse holding for the presumed tissue or insoluble acid. Rienits' data support the working hypothesis that the non-exudate hyaluronic acid affects the water content of the sexual skin by causing the retention, in the extracellular space, of serum protein. The protein then causes oedema by altering the balance of the hydrostatic and osmotic forces controlling the extracellular volume. This, of course, takes us only part of the way, since the hypothesis does not attempt to explain how oestrogen influences the biosynthesis of hyaluronic acid in the sexual skin, or what process releases the insoluble hyaluronic acid into the free fluid, and so leads to the amazingly rapid shift of water from the skin and from the body.

7. Mechanism of the Menstrual Cycle

The dramatic disappearance of oedema from the sexual skin at the time of ovulation is mirrored in an equally dramatic dehydration of the primate endometrium which occurs about a fortnight later, just before menstruation (Markee, 1938, 1940, 1947). Our understanding of the hormonal control of this process begins with Corner's (1923) observation that ovulation is not a prerequisite to normally spaced menstrual bleedings. Haemorrhage can occur either from an endometrium which shows only the proliferative changes characteristic of oestrogenic stimulation, or from one that has also undergone secretory differentiation in the second half of the cycle (ovulation occurring somewhere about its middle) under the influence of progesterone produced by the corpus luteum.

In 1927 Allen discovered that the removal of the ovaries precipitates uterine bleeding in rhesus monkeys, and that bleeding also occurs a few days after the cessation of a course of injections of oestrogen. This observation established the fact that uterine bleeding occurs when some previously acting hormonal stimulus—in this case oestrogen—is withdrawn. It was next found that the uterine bleeding which would otherwise occur a few days after the end of a series of oestrogen injections could be delayed, although not stopped, if the dosage was dropped to a low level (Zuckerman,1937b). In this way it became possible to simulate a succession of menstrual cycles in spayed monkeys. Similarly, it was also found that uterine bleeding never occurred in monkeys that were maintained indefinitely on a high level of oestrogen, and further, that, if readministered soon after the cessation of a previous course of treatment with oestrogen, oestrogen would inhibit the uterine bleeding that would otherwise occur (Zuckerman, 1937c). On the other hand, the hormone could not prevent the bleeding which took place after the endometrium had undergone progestational differentiation, as a result of stimulation by progesterone, whereas progesterone was able to inhibit oestrogen-withdrawal bleeding (Zuckerman, 1937d). More recently, it has been found that progesterone is able to induce uterine bleeding quite independently of any previous action of oestrogen (see Krohn, 1955). So far as the proximate cause of bleeding is concerned, all that can be said is that an important factor may well be a sudden shift of water, and that this may be associated with a change in the vascular architecture of the endometrium.

In the course of this work it was discovered that periodic uterine bleeding continues to recur in spayed monkeys that

are maintained continuously on a threshold dose of oestrogen (Zuckerman, 1938, 1941; Krohn, 1949). It was also found that the endometrium is more responsive to a second oestrogenic stimulus after treatment with a previous high dose of oestrogen, than it is after treatment with a low dose (Eckstein, Krohn, Zuckerman & Healy, 1952). There is no obvious explanation for the latter observation, but the former raises an interesting question. Since oestrogen-withdrawal uterine bleeding occurs only after the intensity of a previous stimulus falls below a critical level, it follows either that the uterus itself undergoes cyclical variations in sensitivity to stimulation, bleeding occurring when it becomes refractory, or that after ovariectomy some non-ovarian tissue, probably the adrenal cortex, secretes sub-threshold amounts of oestrogen continuously but in fluctuating amounts. This endogenous oestrogen would reinforce the effect of the constant level of exogenous stimulation, but its cyclical fluctuation would induce periodic withdrawal effects.

8. Effects of Oestrogen on Behaviour

As already noted, the changes in the water content of the sexual skin are part of a general effect that oestrogens have on the water metabolism of practically all tissues, including the brain. The fact that the nervous system is affected by oestrogen is also reflected in the behaviour changes for which the hormone is responsible. In most female mammals, sexual behaviour does not begin before the first oestrus, and it fails to appear altogether if the animal is spayed in infancy (see Beach, 1948). Moreover, if puberty is induced precociously by treatment with gonadotrophin, the accelerated maturation of the ovary is associated with the emergence of the appropriate sexual responses (Cole, 1936). Correspondingly, a prepubertally castrated male animal will manifest female mating patterns if it is given adequate doses of oestrogen (Ball, 1939). The influence of this hormone is not, however, confined to sexual behaviour alone, for it has been established in the rat that the level of general activity fluctuates with the oestrous cycle, peaks of activity coinciding with the follicular phase of the cycle (Wang, 1923). These fluctuations occur neither in the prepubertal animal nor in the spayed adult. It has also been found that oestrogen exerts an influence on conditioned reflexes and that it may retard or inhibit more complicated learned reactions (Douglas, Hanson & Zuckerman, 1948). Benedek & Rubenstein (1939) suggest that woman becomes extroverted during the follicular phase of the menstrual cycle, in contrast to a period of introversion which coincides with the luteal phase.

9. Control of Oestrogen Activity

The chronaximetric studies of Chauchard (1943), and Herren & Haterius' (1931) observation that central reflex time increases under the influence of oestrogens, suggest that the hormone in some way modifies the relative excitability of the constituent neurones of the nervous centres concerned, either by affecting specific enzyme systems or indirectly through alterations in the distribution of electrolytes. Unfortunately, we know as little about the way such a change could be brought about as we do about any specific action of the hormone at the cellular level. We also know relatively little about the mechanism or mechanisms that control the cycles of oestrogenic activity within the organism. It was originally believed that the periodicity of oestrogenic action was a reflection of the cyclical maturation of follicles in the ovaries. This view had to be abandoned when it was discovered, first, that the ovary was under the direct control of the gonadotrophic secretions of the anterior lobe of the pituitary, and second, when it was demonstrated that oestrous cycles may continue in mice whose ovaries, after *x* irradiation, no longer contain follicles in any stage of development (Parkes, 1926). There is now a considerable body of evidence which suggests that the gonadotrophic potency of the anterior pituitary is modulated by a feed-back mechanism in which the determining factor is the level at which the ovarian hormones are secreted. Thus, as its titre in the blood rises, the oestrogen that is secreted by ovarian tissue when stimulated by follicle-stimulating hormone (FSH) and luteinizing hormone (LH) checks the further secretion of FSH, and, provided that its concentration does not rise too far, stimulates the further release of LH. In high doses oestrogen causes hyperplastic changes in the anterior pituitary, sometimes leading to adenomatous growths. This effect has a number of secondary results, among them being the complete involution of the ovary, due possibly to the suppression of all gonadotrophic secretion, and the inhibition of general body growth (oestrogen may also have a direct effect on the growth of bones). These changes can be regarded as extreme manifestations of a reciprocal and controlling interaction of the ovaries and anterior pituitary. Another controlling mechanism is provided by the interaction of oestrogens with such other hormones as progesterone and cortical hormone (cf. section 7). It is also fairly clear that the effects of oestrogen are modulated by a number of other factors which control tissue metabolism (e.g. vitamin A).

There is an additional view that the timing mechanism that regulates the phasing of the gonadotrophic action of the anterior lobe of the pituitary is provided by some " sex centre " in the hypothalamus (Markee, Everett & Sawyer, 1952). This hypothesis is related to the view that the " reflex " functions of the anterior lobe of the pituitary are regulated by means of chemotransmitters which pass into the sinusoids of the pars distalis via the pituitary-portal vessels. It has, however, been shown that these vessels are neither a sufficient explanation of, nor necessary for, certain of the responses in question (Thomson & Zuckerman, 1954, 1955; Zuckerman, 1955; Everett, 1954). It seems clear that this problem, and that of the manner of action of oestrogen at the cellular level, remain two outstanding issues on which further research is urgently needed.

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ENDOCRINOLOGY OF PREGNANCY

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With the transition from oviparity to viviparity in mammals and also in fishes and reptiles, there have occurred profound changes in the nature of the egg and in the structure and physiology of the female animal, particularly of the reproductive tract. It would be difficult to imagine that these complex and highly integrated changes and events, observed in vertebrates, particularly in reptiles, could have come about otherwise than through many separate lines of evolutionary specialization. Nevertheless, it is clear that none of these evolutionary innovations could have occurred in the complete absence of various interactions between tissues and hormones.

That there have been many specializations in the responses of the reproductive tract to hormones is apparent, but we are still unable to find a unifying concept that fits all the facts, although various groups of vertebrates have been investigated. The correlation of the presence of placental tissue with certain changes and events in man and the lower vertebrates points to the placenta having an endocrine function in controlling pregnancy. "The difficulty", as Newton (1938) remarks, "is to express this in more precise terms and to prove it in detail." Much is known and a great deal is surmised.

The internal secretions that take direct part in initiating, maintaining and terminating pregnancy are those of the ovary and placenta and the gonadotrophic hormones of the pituitary, while other endocrine glands, whose products are more directly concerned with the general metabolism of the body, exercise an indirect though important influence.[1]

[1] See Chester Jones, p. 156 of this number of the Bulletin.—Ed.

1. Role of the Anterior Hypophysis in Pregnancy

a. Effects of Hypophysectomy in Mammals

In the pregnant animal the effects of hypophysectomy vary according to the species and the stage of gestation when it is performed. In some species, e.g. rabbit (Robson, 1937, 1940), dog (Houssay, 1935) and cat (McPhail, 1935), abortion occurs no matter when the operation is performed. In others (e.g. monkey, mouse, guinea-pig and rat) pregnancy is interrupted only if ablation is performed during early pregnancy. In the monkey and mouse, delivery of young occurs at the normal time, and mammary development is complete (Smith, 1946, 1954; Selye, Collip & Thompson, 1935; Newton & Beck, 1939; Newton & Richardson, 1940; Gardner & Allen, 1942), but absence of pituitary function becomes apparent after parturition, when lactation fails to occur (Newton & Richardson, 1940; Smith, 1946). In hypophysectomized mice in which the products of conception are reduced to placentae, full mammary development is sometimes impaired, but body-weight is maintained (Newton & Beck, 1939), luteal function is sustained (Selye *et al.* 1935; Deanesly & Newton, 1940), and the development of the interpubic ligament proceeds normally (Newton & Beck, 1939).

b. Effects of Hypophysectomy in Lower Vertebrates

The results of hypophysectomy in gestating snakes (Clausen, 1940) suggest that the pituitary is an essential structure for the maintenance of normal pregnancy. In early and middle pregnancy it controls the luteal function of the corpus luteum. In late gestation it controls the elaboration of pregnancy-maintaining hormone from placenta-like tissues, as suggested by the results of ovariectomy (Table I). On the other hand, the hypophysis can be dispensed with in the smooth dog-fish until at least the fifth month of its ten months' gestation, without affecting either the development of the embryos, the absorption of yolk or the development of the yolk-sac placenta (Hisaw & Abramowitz, 1937).

c. Relationship between the Anterior Hypophysis and the Placenta

In rats, the anterior pituitary is essential for the establishment and maintenance of pregnancy to the eleventh day. After this time it can be dispensed with and pregnancy will continue (Pencharz & Long, 1931). Furthermore, Lyons (1943) found that gestation will continue in ovariectomized and hypophysectomized rats receiving oestrin (1 mg.) and progesterone (2 mg.). Since the ovaries are necessary in the rat throughout pregnancy (see below), it is assumed that the continuation of pregnancy after hypophysectomy in this species must be dependent on the maintenance of the corpora lutea by luteotrophic principles from an extrahypophyseal source. Astwood & Greep (1938) prepared luteotrophic extracts from rat placenta, and these original findings have been confirmed by Astwood (1941) and by others (see Evans & Simpson, 1950). Moreover, the fact that Deanesly & Newton (1940) found the luteotrophic action of the mouse placenta to be independent of the pituitary, and the further important discovery of Philipp (1930) and others, that in pregnancy the anterior pituitary seems to discontinue the production of gonadotrophin, emphasize the wide functional significance of the placenta.

It must be pointed out, however, that luteal function in hypophysectomized rabbits can be maintained by oestrogenic

TABLE I.* EFFECTS OF OVARIECTOMY AND HYPOPHYSECTOMY ON GESTATION IN SNAKES

Species	Number of cases	Stage of gestation	General results	Autopsy findings
Effects of ovariectomy on gestation				
Water snakes:				
Natrix cyclopion	4	Early	Resorption	No evidence of young
N. cyclopion	5	Middle	Resorption (3 cases)	3 dead embryos in one cage
N. cyclopion	6	Late	Normal delivery of 44 young (total in all 6 cases)	No evidence of any young in tubes
N. sipedon confluens	8	Early	Resorption	No embryos or yolk present
N. s. confluens	4	Middle	Resorption	6–8 hard yolk masses found in each snake
N. s. confluens	8	Late	Normal delivery of live young	3 well-developed dead embryos in one case
N. rhombifera	8	Late	Normal young delivered (7 cases)	In one case 14 mature dead young found
N. s. sipedon	4	Early	Resorption	No evidence of yolk masses or young
N. s. sipedon	3	Middle	Abortion of all dead embryos 6 days after operation	No evidence of young or yolk in tubes
Garter snakes:				
Thamnophis sirtalis	8	Late	Normal delivery of live young	No cases of dead young in tubes
Brown snakes:				
Storeria dekayi	6	Middle	Resorption (4 cases) Abortion (2 cases)	Abortion complete in the 2 cases
Effects of hypophysectomy on gestation				
Water snakes:				
Natrix cyclopion	6	Late	Abortion	2 snakes had 3 yolk bodies in tubes
N. sipedon confluens	4	Early	Resorption	Yolk masses found in all tubes
N. s. confluens	4	Middle	Abortion (all young dead)	No material in tubes
Garter snakes:				
Thamnophis butleri	4	Early	Resorption	6–9 yolk masses in each animal
Brown snakes:				
Storeria dekayi	4	Early	Resorption	1 case had partially developed young in tubes
S. dekayi	6	Late	Abortion—61 dead embryos	No evidence of yolk or embryos in tubes

* From Clausen (1940, Table I).

substances—as well as by the administration of gonadotrophic hormones (Robson, 1937, 1938), and it has been suggested that, at least in the rabbit, gonadotrophic hormones have no direct action on luteal function, but further luteal activity by stimulating oestrogen production in the ovary (Robson, 1947). In this connexion it should also be mentioned that Williams (1940) has shown that oestrogens have a direct action upon the ovary and can bring about ovarian hypertrophy in the hypophysectomized rat.

The foregoing considerations naturally raise the question of the existence of two separate controlling mechanisms for the maintenance of the corpus luteum: the one involving the direct action of gonadotrophic hormone on the corpus luteum, the other an oestrogenic substance. But, here again, it is likely that species differences exist in the relative importance of the ovarian and pituitary hormones in this regard.

2. Endocrine Role of the Ovary

a. Effects of Ovariectomy in Mammals

Since both oestrogen and progesterone are essential for the full differentiation of the endometrium, removal of the ovaries before this condition has been established prevents implantation of the blastocyst and placentation. As with the pituitary, however, there are species differences with respect to the importance of the ovary for the maintenance of pregnancy.

The ovary has been found necessary for the maintenance of pregnancy throughout most, if not all, of gestation in the opossum (Hartman, 1925), golden hamster (Klein, 1938), 13-striped ground-squirrel (Johnson & Challans, 1932), mouse (Parkes, 1928; Robson, 1936), rat (Hain, 1932; McKeown & Zuckerman, 1938; Selye *et al.* 1935), rabbit (Corner, 1928), goat (Drummond-Robinson & Asdell, 1926), and cow (Hammond, 1927). On the other hand, in the ewe (Cassida & Warwick, 1945), mare (Hart & Cole, 1934), bitch (Marshall & Jolly, 1906), cat (Gros, 1936; Amoroso, unpublished data, 1942), guinea-pig (Loeb, 1923; Rowlands, personal communication, 1954), monkey (Hartman, 1941), and woman (Asdell, 1928), pregnancy can proceed normally in the absence of the ovaries after a certain stage of gestation has been reached. In women, monkeys and mares, ovariectomy may be performed during the early months of pregnancy without apparently interrupting its normal course, whereas in the guinea-pig, cat, dog and sheep, pregnancy is only regularly maintained when the operation is performed during the second half of gestation. But even in those species in which ovariectomy normally terminates pregnancy, the reduction of the conceptuses to a minimum (Harterius, 1936), or the retention of one or more placentae (the foetuses having been removed), will result in delivery of the retained foetuses or placentae at term (Kirsch, 1938; Newton, 1935; Huggett & Pritchard, 1945a, 1945b; and others), emphasizing once again the importance of the placenta in the maintenance of pregnancy.

b. Effects of Ovariectomy in Lower Vertebrates

Clausen (1940) studied the effects of ovariectomy on gestation in three species of viviparous snakes (Table I), and concluded that the corpora lutea are of considerable importance in snakes in the early and middle stages of gestation and of less importance in the later stages. He suggests that the corpora lutea, under the influence of the pituitary, elaborate

a hormone necessary for the completion of the early stages of pregnancy, whereas the failure of abortion after castration in late stages, he believes, may be due to endocrine secretions from tissues analogous to the placenta in mammals. Interesting in this connexion is the fact that progesterone has been extracted from the ovaries of ovoviviparous snakes (Valle & Valle, 1943), and that progesterone-like agents have also been demonstrated in the circulating plasma (Bragdon, Lazo-Wasem, Zarrow & Hisaw, 1954). Consequently, the failure of exogenous progesterone to repair the effects of ovariectomy in early pregnancy in these species (Clausen, 1940) may imply that, for the restoration of full ovarian function, oestrogen is required as well. In viviparous elasmobranchs, the ovaries are apparently not essential for the maintenance of pregnancy.

These experiments together with the foregoing ones on the effects of hypophysectomy suggest, therefore, that the placenta may maintain the activity of the ovaries during pregnancy in some animals; and there is, moreover, evidence that the presence of the pituitary is not necessary in order that the action of the placenta on the ovary and the corpus luteum may be produced (Newton & Beck, 1939; Deanesly & Newton, 1940; Clausen, 1940).

3. Relationship Between the Ovary and the Placenta

a. Oestrogenic Substances

As far as is known, very little or no oestrogenic material is produced in organs other than the ovaries, adrenals, placenta and testes.

It is agreed by all workers that urinary excretion of oestrogen increases during the course of pregnancy (Cohen, Marrian & Watson, 1935; Hain, 1940; Venning, 1946, 1948; Bradshaw & Jessop, 1953), and that, in the pregnant mare and woman, double ovariectomy does not abolish the urinary excretion of these substances (Hart & Cole, 1934; Allan & Dodds, 1935; and others). Also Dorfman & van Wagenen (1941) found the removal of ovaries and foetus ineffective in altering the excretion rate in pregnant monkeys, but expulsion of the placenta caused a drop to non-pregnant levels. These facts suggest that the oestrogens are released from some extra-gonadal source, and the balance of evidence available at present strongly indicates that the placenta is mainly responsible for the production of the huge amounts of oestrogens found in human, primate and equine pregnancy.

Marrian (1938) has shown that oestrogens may be excreted in a variety of ways. The amounts of " combined " oestrone and oestriol excreted diminish shortly before parturition, while the proportion of active free oestriol shows a correspondingly sharp rise. It is noteworthy that during the greater part of pregnancy over 99% of the total urinary oestrogen is in the form of oestriol glucuronide and that such conjugated oestrogens have little physiological potency (Clayton & Marrian, 1950). It is fairly clear, therefore, that oestrin has a function during parturition which it is prevented from exercising in the preceding months of pregnancy.

b. Site of Production of Oestrogenic Steroids during Pregnancy

The best indirect evidence in favour of oestrogen production by the placenta is provided by Stewart (1951), who demonstrated oestrogenic stimulation of the uterus in ovariectomized rabbits with intra-ocular implants of full-term human placenta. Moreover, the histological examination of the implants established the occurrence of syncytium, which Dempsey & Wislocki (1944) and Wislocki & Dempsey (1948) believe to be in some way associated with the synthesis of the steroid hormones.

c. Effects of Oestrogens on the Foetal Gonads

These have been discussed by Amoroso & Rowlands (1951), and the evidence points to the existence of an association between the concentration of urinary oestrogens in the pregnant mare and the quantitative development and regression of the foetal gonads. Similar quantitative changes have been observed in the gonads of the seal at birth, but as yet no correlation between urinary oestrogens and gonadal development has been reported (Amoroso, Harrison, Matthews & Rowlands, 1951; Amoroso & Matthews, 1951; Amoroso & Matthews, 1952).

d. Corpus Luteum

John Beard (1897) was among the first to suggest that the corpus luteum constituted an organ of pregnancy. In vertebrates these luteal structures are not invariably associated with viviparity, since they have been described in oviparous and ovoviviparous selachians, teleosts and reptiles (Giacomini, 1895; Cunningham & Smart, 1934; Hisaw & Abramowitz, 1937; Rahn, 1938, 1939; Clausen, 1940; Cieslak, 1945; Bretschneider & de Wit, 1947), and in mammals, where they undergo their greatest specialization, they first appear in the oviparous Monotremes.

In oviparous and ovoviviparous teleosts the corpus luteum is of the pre-ovulatory type. It has been shown to produce a secretion, " oviductin " (Bretschneider & de Wit, 1947), which is responsible for the growth of the ovipositor in the bitterling, thus providing the first direct evidence that the secretory function of the corpus luteum is not an evolutionary innovation of the mammalia. It must be pointed out, however, that while progesterone is not the effective secretion of the corpus luteum in the bitterling, it is outstandingly effective in inducing the rapid elongation of the ovipositor in this species.

In mammals, experimental proof of the importance of the corpus luteum in pregnancy was provided by Loeb (1923), Drummond-Robinson & Asdell (1926) and Hammond (1927), when they showed that removal of this organ, in the guinea-pig, goat and cow respectively, was soon followed by ovulation, regardless of whether the products of conception are expelled immediately or retained temporarily. Additional evidence that the premature occurrence of ovulation following removal of the corpus luteum is due to withdrawal of the active principle progesterone was provided by Parkes & Bellerby (1927) and by Selye, Browne & Collip (1936).

e. Pre-Ovulatory Progesterone

On the basis of indirect histological evidence (pre-ovulatory luteinization), it has repeatedly been implied or suggested that progesterone may be secreted shortly before or at the time of ovulation (Harrison, 1948), and progesterone has been detected by various tests in the follicular fluid of sows and cows and in the peripheral blood of the rabbit, ewe and sow (Forbes, 1953; Edgar, 1952, 1953a, 1953b).

f. Biology of Progesterone

That progesterone plays an important part in conditioning uterine reactions during pseudopregnancy and during

pregnancy is one of the elementary facts of physiology that is learned by every medical or veterinary student during his first year of training. There is, however, a good deal of evidence (summarized by Courrier, 1950) that many of the effects produced by the luteal hormone under physiological conditions involve the simultaneous action of the oestrogenic hormone as well.

Marrian (1929) was the first to obtain pregnanediol from human pregnancy urine. This reduction product, which is inactive as a progestin, occurs in conjugation with glucuronic acid (Venning & Browne, 1937) and has not, as yet, been found in the urine of any species other than man. Progesterone metabolism may, therefore, not follow an identical course in all mammalian species.

Venning (1938), Hain (1940) and others have studied the curve of pregnanediol excretion throughout pregnancy.[2] After the first two months of pregnancy the excretion rate rises progressively, but falls precipitously at parturition. Likewise, Jones & Weil (1938), having obtained the pregnanediol complex from the urine of a pregnant woman following ovariectomy on the fifty-eighth day after the last menstrual flow, concluded that progesterone is produced during early pregnancy by the corpus luteum, whereas an additional supply is provided by the placenta after the second month.

We have already alluded to the isolation of progesterone from the adrenal glands and corpus luteum and have also commented on the observation of Harterius (1936), Newton (1938) and others, that pregnancy may be maintained in the rat after bilateral oophorectomy, if most of the foetuses are removed and the placentae retained, thus assuring a high placental–foetal ratio. More recently progesterone has been isolated from human placenta (Pearlman & Cerceo, 1952; Noall, Salhanick & Zarrow, 1952), and its occurrence in the equine placenta is strongly suspected.

More progesterone is normally secreted by the corpus luteum than is necessary to maintain gestation, for example, in rabbits (Corner, 1937), and goats (Meites, Webster, Young, Thorp & Hatch, 1951). In ovariectomized rabbits the complete maintenance of pregnancy requires a dosage of 1 mg./day during the first 10–12 days and 2–3 mg./day thereafter (Pincus & Werthessen, 1938; Courrier & Kehl, 1938; Allen & Heckel, 1939). Mice require 1–1.5 mg. of the hormone (Robson, 1938). In sheep it is possible that from 5 to 10 mg. represent the daily requirement (Dutt & Cassida, 1948); for the goat 10 mg. daily suffice to maintain pregnancy during the last third of pregnancy. It is interesting that pregnant women, whose average body-weights fall within the same range as pregnant goats, have been shown to secrete the equivalent of 60–100 mg. of progesterone daily during the last third of pregnancy (Corner, 1941). Recent observations by Raeside & Turner (1950a, 1950b) indicate that pregnant cows, which may weigh eight times as much as goats or women, require only 50–75 mg. of progesterone daily to maintain pregnancy after removal of the corpus luteum. Available evidence indicates that less progesterone is necessary to maintain pregnancy when given with oestradiol, than when given alone (Courrier & Jost, 1939).

g. Progesterone and Myometrial Function

Detailed studies of the isometric tension developed by uterine muscle dominated by oestrogen and progesterone, in isolated strips or in intact animals, have been reported by Csapo (1948), Corner & Csapo (1953) and Schofield (1954). The pattern of contractility was found to vary according to whether the dominating hormone was oestrogen or progesterone, the progesterone-dominated uterus developing less tension when stimulated than the oestrogen-dominated uterus. It appears that the effect of the ovarian hormones, oestrogen and progesterone, on the smooth muscle of the uterus is accomplished by alteration of the potassium balance of the myometrial cell.

[2] See also Venning, p. 140 of this number of the Bulletin.—Ed.

4. Pelvic Adaptations and Pubic Separation in Mammals

In some fossorial Insectivora (e.g. mole), where the pelvis is greatly reduced in adults (Wood, 1859), marked differences in pelvic structure have been demonstrated at different developmental ages (Hisaw & Zilley, 1927).

Figure 1 illustrates how the pubic cartilage of the embryo of the American mole (*Scalopus aquaticus machrinus*) becomes resorbed in both sexes soon after birth and how the two halves of the pelvis join to form a secondary pelvis dorsal to the digestive tract. Thus, in the adult the pelvic cavity and outlet are so straight that the pelvic viscera pass below and external to the cavity instead of through it, making it unnecessary for parturition to take place through a constricted birth canal.

The arrangements just described are clearly adaptive, and involve no apparent endocrine mechanisms. On the other hand, skeletal adjustments involving both the symphysis pubis and the sacro-iliac union that are definitely conditioned by hormones have been described in the pocket gopher (Hisaw, 1925), guinea-pig (Hisaw, 1926, 1929; Ruth, 1937; Talmage, 1947) and mouse (Gardner, 1936; Hall & Newton, 1946a, 1946b; Hall, 1947). In these animals, there is a sexual dimorphism of the pelvis and the symphysis pubis reacts to oestrogens and to relaxin as a secondary sexual character.

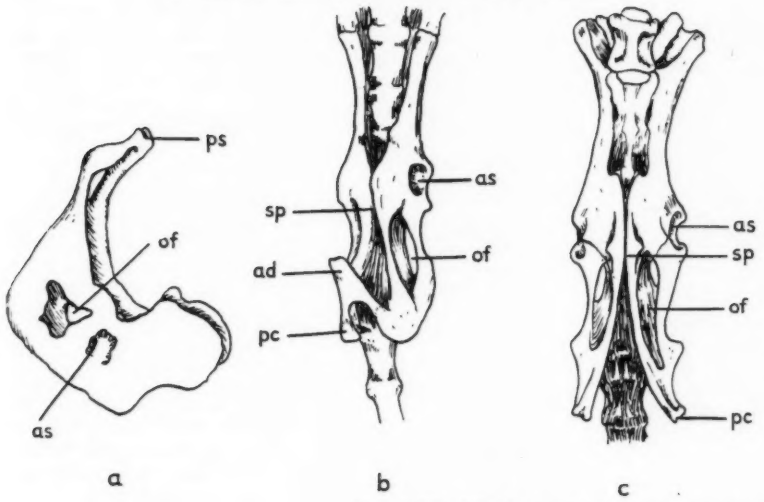
The hormone mainly responsible for these changes in the pelvis has been prepared in a relatively pure state (Abramowitz, Money, Zarrow, Talmage, Kleinholz & Hisaw, 1944) and found to be associated with the pseudoglobulin fraction of serum and to have a peptide-like structure, though the N content of 11% was rather low. Preparations of relaxin are devoid of oestrogenic or progestational activity.

The ovary, uterus and placenta have been suggested as possible sites for the formation of relaxin. In the sow the ovary would appear to be the principal source (Hisaw & Zarrow, 1948), whereas the placenta may play a more significant role in the mouse (Newton & Beck, 1939; Hall & Newton, 1947) and guinea-pig (Herrick, 1928; Zarrow, 1948). Finally, in the rabbit, under suitable conditions, the ovaries, uterus, placenta and vagina may all secrete relaxin (Zarrow & Rosenberg, 1953).

Newton and his collaborators are chiefly responsible for the view that the formation of relaxin in the mouse is apparently different from that observed in guinea-pigs and rabbits. In this animal hypophysectomy during pregnancy does not influence pubic separation, provided that the placentae are not lost, so that a relationship between placenta and ovary is involved (Newton & Beck, 1939). Hall & Newton (1947) suggest that in the mouse the placenta acts by stimulating the production of relaxin in the ovary, probably by means of a luteotrophic hormone.

The claim of relaxin to individuality rests on its speed of action (Table II); its chemical difference from the other two hormones, oestrogen and progesterone; its lack of

FIG. 1. COMPARISON OF THE PELVIS IN AN EMBRYONIC, A HALF-GROWN, AND AN ADULT MOLE



Hisaw & Zilley (1927) by permission of Journal of Mammalogy

a: a reconstruction of the pelvis of a 20 mm. mole embryo

b: pelvis of a half-grown mole (cartilage is shown by stippling, and bone by lines)

c: pelvis of an adult mole

as: acetabulum
of: obturator foramen
ps: pubic symphysis
ad: rectus abdominis muscle
pc: unresorbed base of pubic cartilage
sp: position of secondary pubic symphysis

TABLE II.* TIME REQUIRED FOR PUBIC RELAXATION IN THE CASTRATED, AND CASTRATED AND HYSTERECTOMIZED, GUINEA-PIG AFTER TREATMENT WITH OESTRADIOL, PROGESTERONE AND RELAXIN

Treatment (daily)		Relaxin (g.p.u.)†	Average relaxation time (days)
Oestradiol (μg.)	Progesterone (mg.)		
Castrated			
10	1 (from 11th day)	—	13.5
10	—	—	23.7
1	—	2‡	4.0
Castrated and hysterectomized			
10	1 (from 11th day)	—	23.7
10	—	—	25.6
1	—	2‡	4.0

* Based on Table II of Zarrow (1948).

† g.p.u. = guinea-pig unit.

‡ The relaxin was injected in one dose on the 4th day, after priming the animals with oestrogen for 3 days.

oestrogenic or progestational effect; its action in the hysterectomized animal; and its ability to bring about pubic separation by active bony resorption and ligament formation.

5. Ovarian Activity and Hysterectomy

The fact that the endocrine functions of the ovary dominate the uterus and vagina is well known to many biologists. What is not so generally known, nor so fully understood, is that the uterus exercises an influence on the ovary through the corpus luteum. The evidence from hysterectomy is, however, equivocal and will only be summarized here. Thus, in the pseudo-pregnant guinea-pig (Loeb, 1923); rabbit (Asdell & Hammond, 1933) and rat (Bradbury, 1937), hysterectomy prolongs the life-span of the corpus luteum. On the other hand, even partial hysterectomy in women may sometimes be followed by a premature menopause. In the pregnant guinea-pig (as in most mammals), removal of the products of conception is followed by regression of the corpus luteum; whereas removal of the uterus, as well as of its contents, lengthens the life-span for two months or longer (Desclin, 1932; Klein, 1939). These studies should be repeated. It has been suggested (Heckel, 1942) that hysterectomy exerts a sparing effect on endogenous oestrogen, which then becomes available in quantity and is utilized for prolonging the life-span of the corpora lutea (Robson, 1937; see also section 1c of this paper, and Pincus, 1937).

6. Placental Gonadotrophins

It is generally believed that the placenta releases gonadotrophins similar to—but physiologically and chemically different from—those elaborated by the anterior hypophysis. These placental products, as found in the body fluids of a variety of mammals, have been shown to differ markedly in their physiological properties in the different species.

a. Human Chorionic Gonadotrophin

In the human female these substances are elaborated in the foetal chorion (see below) and appear in the blood and urine within two weeks of fertilization, reaching their highest concentration in the second and third lunar months. There is an abrupt drop in the fourth month, and thereafter the production, utilization and elimination of the hormone remain fairly constant until term (Albert & Berkson, 1951; Diczfalusy, 1953; and others). Lyons, Simpson & Evans (1953) have demonstrated qualitative changes in the urinary gonadotrophins. In primates (chimpanzee and monkey), the elimination of chorionic gonadotrophin ceases before the end of pregnancy and is limited to a brief period at its beginning (Zuckerman, 1935; Hamlett, 1937). The excretion rates of various hormones in pregnancy are given by Selye (1949) and by Venning (1946).

b. Gonad-Stimulating Hormone in Giraffe

The presence of a gonad-stimulating substance has been reported in the urine of the pregnant giraffe (Wilkinson & de Fremery, 1940) and it would appear that its elimination in the urine ceases before term (Amoroso & Kellas, unpublished data, 1954). As judged by the extensive follicular development and luteinization in the foetal ovaries, a relationship between hormone production and foetal gonadal activity in this species is suspected (Plate I, fig. A).

c. Pregnant Mare Serum Gonadotrophin

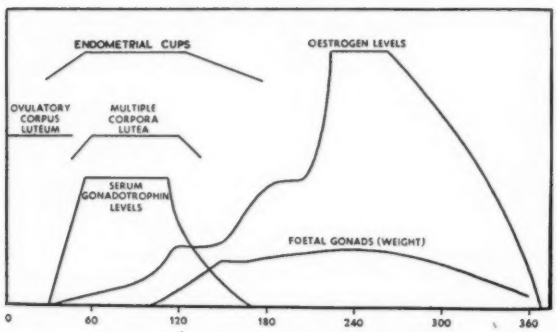
A potent gonadotrophin has also been demonstrated in the blood of pregnant mares (Cole & Hart, 1930; Day & Rowlands, 1940; Day & Rowlands, 1947; Rowlands, 1949). Day & Miller (1940) suggest that probably all mares secrete a gonad-stimulating hormone in early stages of pregnancy. Its appearance in the blood and in the lymph corresponds to the time of implantation of the blastocyst (Amoroso, 1952), and it appears to be formed in the tissues comprising the endometrial cups (Cole & Goss, 1943; Rowlands, 1947; Amoroso, 1952; Clegg, Boda & Cole, 1954). The concentration of gonad-stimulating substances in the serum is, in general, inversely proportional to the size of the mare (Cole, 1938; Rowlands, 1949), and is uniformly higher when twins are present (see Amoroso, 1952). The part it plays in gestation is not clear, but it may be significant that its presence is associated with the development and growth of numerous accessory corpora lutea (Amoroso, Hancock & Rowlands, 1948). These accessory structures have completely regressed by about the 180th day and from then until the end of pregnancy the ovaries contain neither corpora lutea nor large follicles. Consequently, the ovaries cannot be regarded as providing a continuing source of progesterone in this species. The events during gestation in the mare are portrayed diagrammatically in fig. 2.

Accessory corpora lutea have likewise been discovered in the ovaries of a pregnant nilgai (*Boselaphus*) bearing twin foetuses (Plate I, fig. B), and Perry (1953) records several generations in the African elephant.

d. Source of Human Chorionic Gonadotrophin

The evidence is ordinarily considered adequate that the origin of the gonadotrophic hormone in the blood and urine of pregnant women is from the placenta, more exactly from the Langhans' cells of the foetal chorion. The earlier evidence for this view, the gonadotrophic activity of placental extracts (Collip, 1930), has been strengthened greatly by the histochemical investigations of Wislocki & Bennett (1943) as well as by the demonstration of gonadotrophic activity in cell-free filtrates of early human placenta cultivated in vitro (Stewart, Sano & Montgomery, 1948) and by the demonstration of gonadotrophic secretions in intra-ocular implants of mature placentae in rabbits (Stewart, 1951).

FIG. 2. THE EVENTS DURING GESTATION IN THE MARE



Abscissae: days of gestation

e. Source of Pregnant Mare Serum Gonadotrophin

The source of mare serum gonadotrophin has not been definitely established, but the evidence points to the endometrial cups being implicated (Cole & Goss, 1943; Rowlands, 1947; Amoroso, 1950, 1951, 1952; Clegg *et al.* 1954). This evidence is as follows: (i) the appearance of the hormone in the blood and lymph is coincident with the development of the cups; (ii) when first formed at 45 days, the cups contain higher concentrations of the hormone than are found in the blood and lymph; and (iii) gonadotrophins can be extracted from the endometrium only in the cup area.

The detailed histology of the cups is illustrated on Plates II, III, IV (figs. C–T) which must serve to amplify the descriptions already given by Amoroso (1952) and Clegg *et al.* (1954).

7. Placental Hormones and the Evolution of Viviparity

It would be extraordinary if the apparent relationship between the facts so far established should prove fortuitous. On the contrary, it does appear that the placenta, through its secretory activity as an accessory gland of pregnancy, holds a key position in determining the endocrine framework within which gestation occurs.

The first step in the evolution of viviparity in mammals has been merely the retention of the embryos in the uterus. In the marsupial cat, *Dasyurus viverrinus* (O'Donoghue, 1912; Hill & O'Donoghue, 1913) and the Virginian opossum, *Didelphis virginiana* (Hartman, 1923), in which pregnancy lasts about 12 days and in which the luteal phase of the oestrous cycle is so prolonged as to be comparable with true pregnancy in duration (complete pseudopregnancy), parturition is correlated with the involution of the corpora lutea. As the ovaries are necessary throughout gestation in these species (Hartman, 1925), it is assumed that the maintenance of pregnancy does not involve hormonal mechanisms other than those that participate in the regulation of the oestrous cycle. In higher mammals, on the other hand, it is clear that there are controlling mechanisms which make it possible for the uterus to retain developing embryos for a longer time than the limits of the oestrous cycle.

The known sources of gonadotrophin furnish rather interesting comparative data. In the rat and probably in all animals in which ovariectomy terminates pregnancy (e.g. hamster, mouse, rabbit), the stimulation of luteal secretion is the only effect known to be produced by this hormone. In the mare, its presence in the serum and lymph is correlated with the development and growth of numerous accessory corpora lutea (the same may be true of the elephant and nilgai). In the human female the principal property of the hormone is one of luteinization, and there is some evidence which indicates that it can stimulate the secretion of both oestrogen and progesterone by the corpus luteum (Hisaw

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(FIGS. A—T)

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FIG. A. Hormonal effects in the ovary of the giraffe foetus. Note presence of follicles and extensive luteinization
(magnification × 7.5)

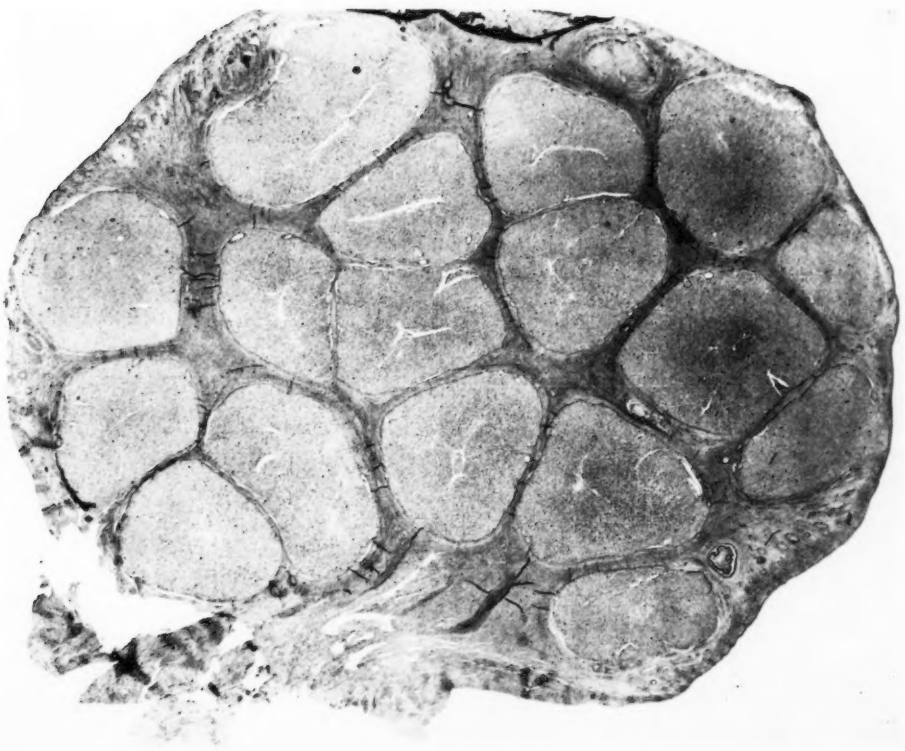

FIG. B. Accessory corpora lutea in the ovary of the nilgai (*Boselaphus tragocamelus* Pallas)
(magnification × 6)

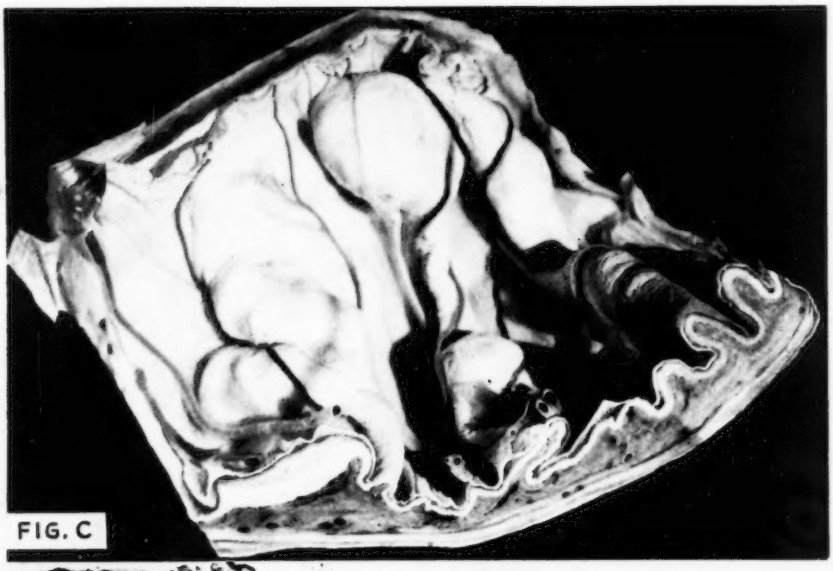

FIG. C

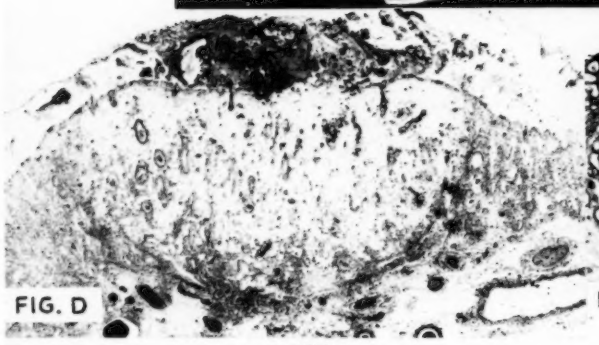

FIG. D

FIG. E

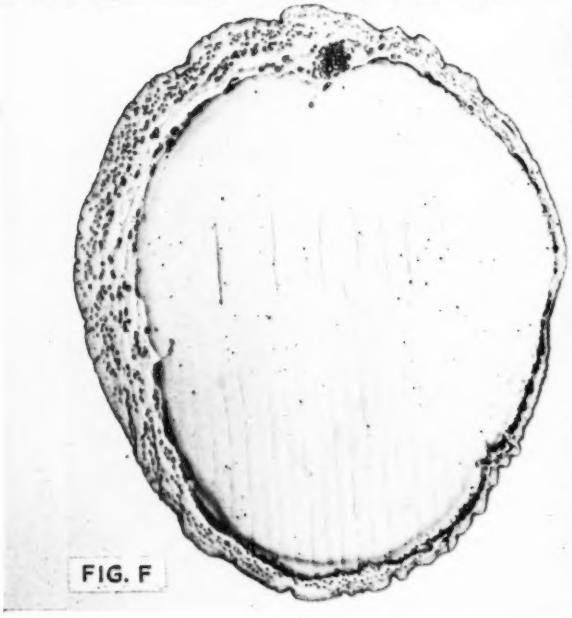

FIG. F

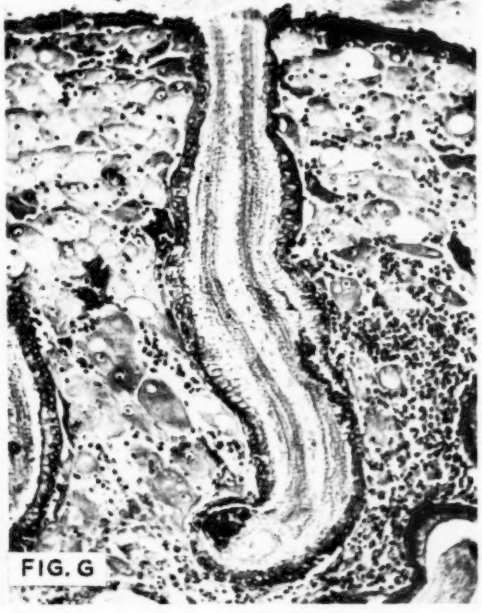

FIG. G

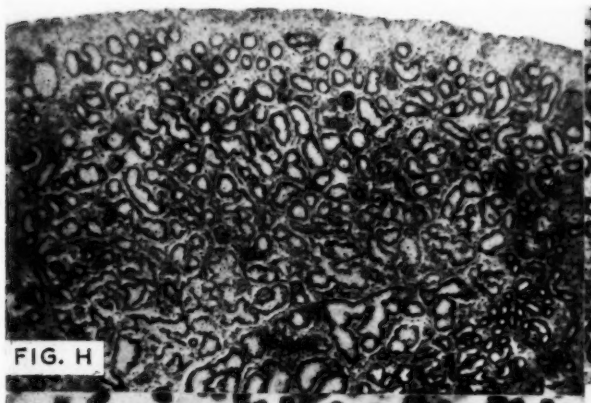

FIG. H

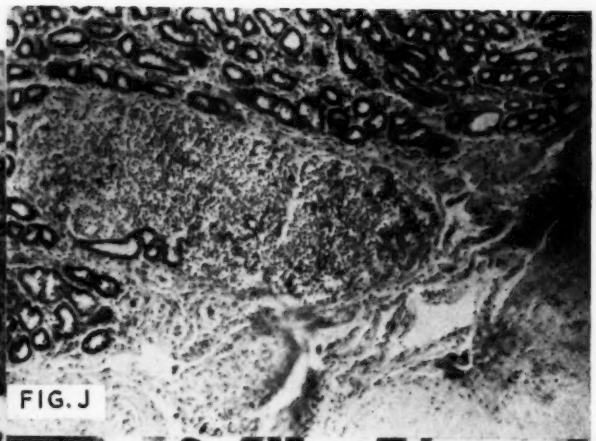

FIG. J

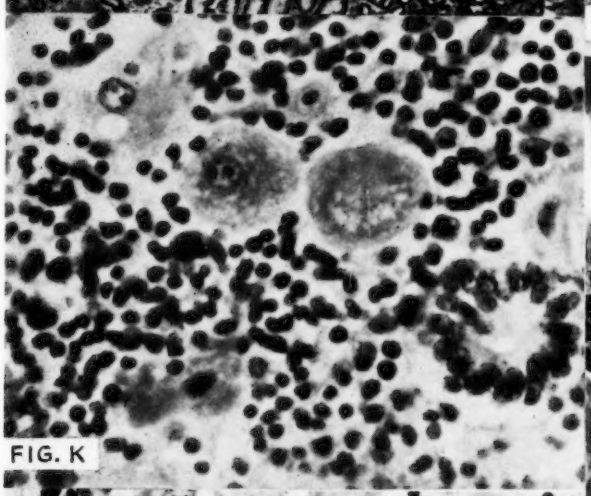

FIG. K

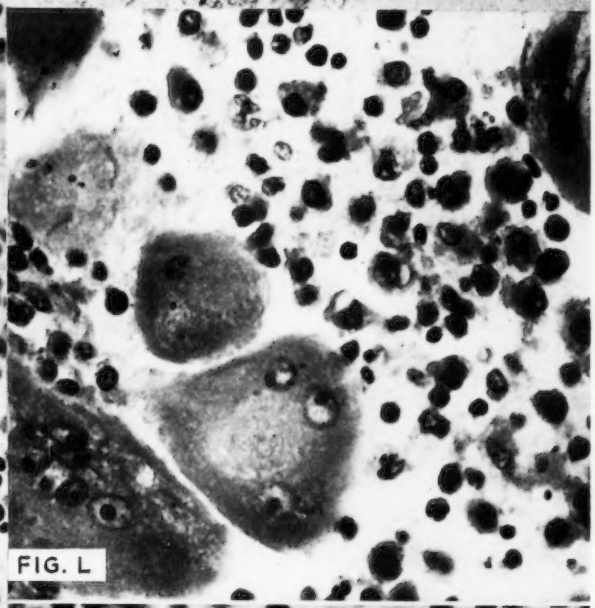

FIG. L

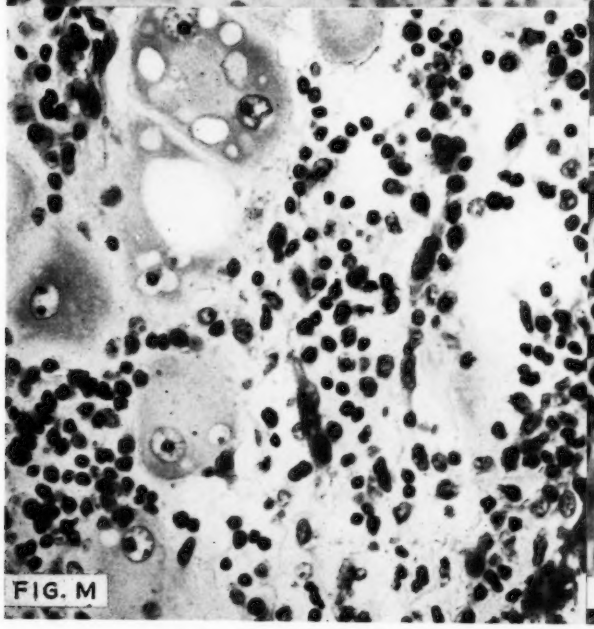

FIG. M

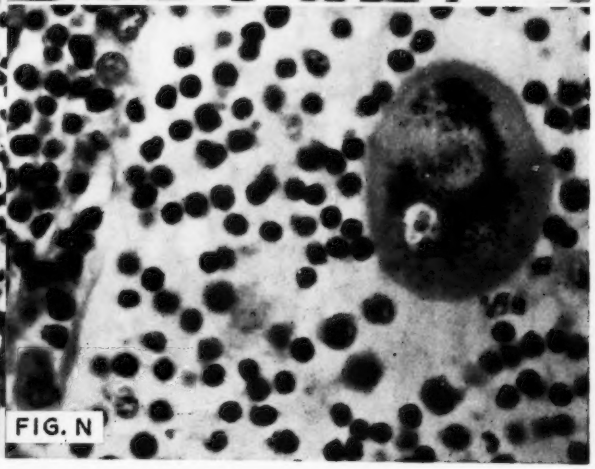

FIG. N

FIG. O

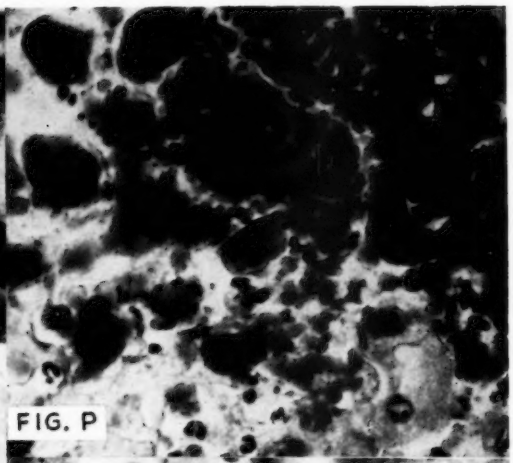

FIG. P

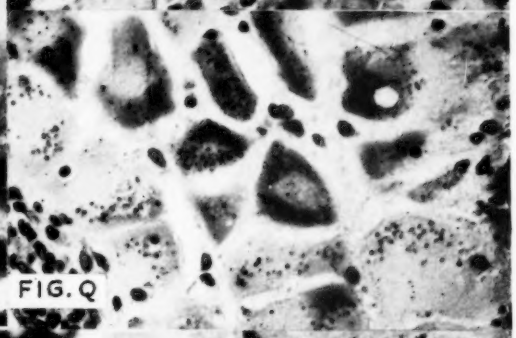

FIG. Q

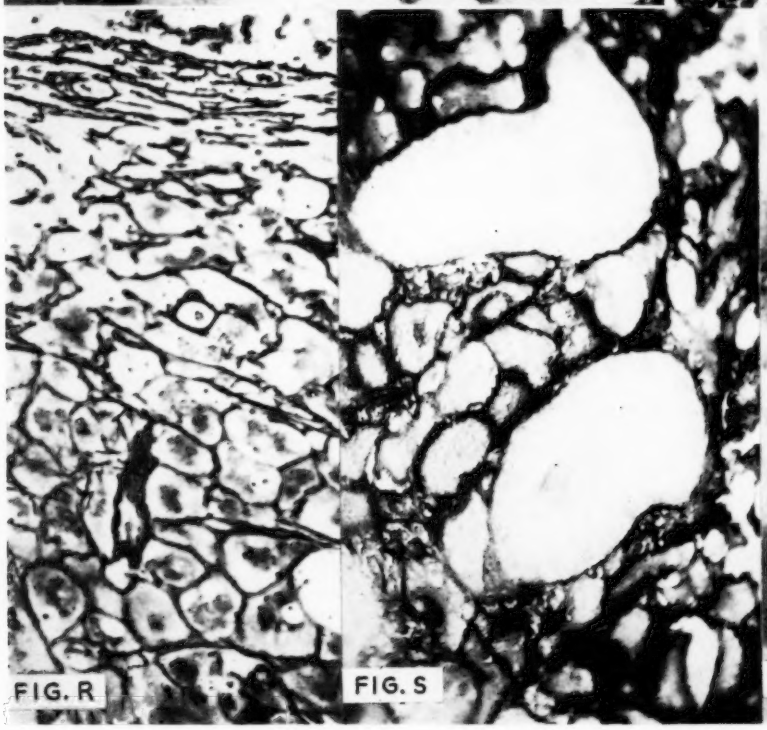

FIG. R

FIG. S

FIG. T

PLATE II. FIGS. C—G

FIG. C. Portion of the interior of the uterine horn, showing endometrial cups, from a mare 63 days pregnant. Note the sectioned surface of a cup and the secretions (uterine milk) filling the depression of the cup. The endometrial cups are limited to the fertile horn and occupy a circular area of the endometrium in the neighbourhood of the yolk-sac. Here the cups develop most frequently in apposition to the chorion which overlies the sinus terminalis
(magnification × 2)

FIG. D. Photomicrograph of a section of an endometrial cup at 50 days. When first formed, the cups have flat surfaces and are sharply delimited from the submucosal tissues by condensations of lymphoid tissue. The viscous coagulum which has accumulated in the pocket of the chorion overlying the cup is derived in part from cytolysing endometrial cup cells and in part from glandular secretions
(magnification × 20)

FIG. E. Section of a fully developed endometrial cup from which the detritic coagulum has been removed. The lip of the depression results in part from overgrowth of the margin of the transformed area and partly from the degeneration and desquamation of the central region of the cup. Note the dilated fundic parts of the uterine glands
(magnification × 10)

FIG. F. Transverse section through the dome of chorion overlying a cup together with its contained secretion. The gonadotrophic activity of this secretion has been shown to be as great as 8,000 i.u./g. of fresh tissue
(magnification × 12)

FIG. G. Section through margin of a cup showing details of mature endometrial (decidual) cells and secretion pouring from a dilated uterine gland. Note the large number of small round cells
(magnification × 150)

PLATE III. FIGS. H—N

FIG. H. Early developing endometrial cup, from a mare 45 days pregnant. In the evolution of an endometrial cup, the decidual reaction is foreshadowed by the degeneration of strictly localized groups of the endometrial glands. Note the paucity of surface crypts and the dilated fundic parts of the glands
(magnification × 45)

FIG. J. Later stage in the development of the cup from the same animal as in fig. H. As the glandular degeneration progresses, there is an accompanying infiltration of small round cells which soon replaces the glandular tissue. Note the extensive obliterating endarteritis in the vessels at the base of the transformed area
(magnification × 77)

FIG. K. Portion of the area in fig. J shown at higher magnification. In the vicinity of the degenerating glands, the stromal elements undergo enlargement and are at first indistinguishable from plasma cells. Some cells are further advanced in development than others
(magnification × 430)

FIG. L. Details of mature endometrial cup cells and transitional forms. When fully developed the cup cells are greatly enlarged and have rounded contours, granular cytoplasm and vesicular nuclei. Binucleate and multinucleate forms are frequently encountered. It is noticeable that transitional forms between small round cells, plasma cells and typical decidual cells continue to appear even in the most mature cups
(magnification × 550)

FIG. M. Section through base of mature endometrial cup, showing relationship of cup cells to lymphatic sinus. The profusion of lymphatic tissue and lymphatic vessels in the neighbourhood of the cups constitutes important identifying characteristics of the decidual reaction
(magnification × 450)

FIG. N. High-power view of part of a lymphatic sinus, showing endometrial cup cell within the lumen of the sinus
(magnification × 540)

PLATE IV. FIGS. O—T

FIG. O. High-power view of part of a fully developed endometrial cup, showing the accumulation of colloid-like material within some of the cells. These colloid droplets, which react positively to leucofuchsin stain, are quite discrete at first. Later they become confluent and in doing so may completely replace the nucleus and cytoplasm.
(magnification × 675)

FIG. P. Surface of an endometrial cup showing the final stages in the degeneration of the cup cells. Many of the cells are now completely filled with colloid and have become detached from the surface of the endometrium, contributing to the coagulum which accumulates in the cavity of the cup and which is rich in gonadotrophin and stains positively with leucofuchsin stain
(magnification × 300)

FIG. Q. Endometrial cup cells showing irregular disposition of pigment
(magnification × 350)

FIG. R. Section through surface of a mature cup, showing disposition of reticular fibres in relation to each cup cell
(magnification × 300)

FIG. S. Endometrial cup showing disposition of alkaline phosphatase. Note that the enzyme is abundant but is confined to the margins of the cells and is absent in the glandular epithelium
(magnification × 300)

FIG. T. High-power view of cup cells stained with Sudan black. After regression of the cup has begun, the cup cells show an increasing infiltration with sudanophilic granules, which are especially large and numerous in the degenerating surface cells and in the detritic mass filling the cup
(magnification × 675)

& Astwood, 1942). An important paper by Hisaw (1944) provides a hypothesis of the relationship between placenta and ovaries in primates. He postulates for the monkey a chain of hormone combinations leading up to the complete dominance of the placenta.

The fact that the secretion of these substances from the three groups of mammals represented is initiated at the time of implantation or attachment of the blastocysts seems to imply that they may, all of them, be instrumental in furthering luteal function. On the other hand, the extent to which the placenta has adopted ovarian functions varies considerably from species to species. After removal of the ovaries in some of these animals, e.g. mouse and rabbit, pregnancy may be maintained by administering progesterone alone (Hall & Newton, 1946b; Allen & Corner, 1929; Corner, 1937; Courrier & Kehl, 1938; Pincus & Werthessen, 1938; Allen & Heckel, 1937), but in the hamster and rat it is necessary to provide oestrogen in addition (Klein, 1935, 1937, 1938; but see also Alexander & Fraser, 1954), while, in the human, monkey and mare, substitution therapy is unnecessary after a certain stage. The implication is that while oestrogen and progesterone are necessary for the maintenance of pregnancy in all animals, in the last group they may both be supplied from elsewhere than the ovaries, and it is generally held that the placenta represents the new source. In another group, which includes the mouse and rabbit, oestrogen but not progesterone may be supplied from this extra-ovarian source, and finally in the hamster and rat, which represent yet another group, neither of these substances can be supplied in sufficient quantities in the absence of the ovaries. That this represents a phylogenetic series may be questioned, but it does give the placenta an important claim to be considered as having decisive effects in extending and maintaining pregnancy. Indeed, the probability, that in all eutherian mammals the placenta secretes a luteotrophic principle at the time of implantation, seems to imply that this was the initial adaptation which made it possible to prolong the life of the corpus luteum, thus extending the gestation period beyond the limits of the cycle; while the apparent influence of the placenta in deputizing for the ovary or in forming accessory corpora lutea through a secondary effect on the ovaries might be regarded as a further placental specialization allowing a still longer gestation period. We do not yet know, of course, but, admitting the correctness of these assumptions, it might be supposed that elephants do it this way. But that is as far as speculation can well go in the present state of our knowledge.

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BIOCHEMISTRY OF THE GONADAL HORMONES

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1 Isolation and chemistry
  a Excretory products
  b Original hormones
2 Occurrence in the body
3 Genesis
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6 Metabolism in vivo
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1. Isolation and Chemistry

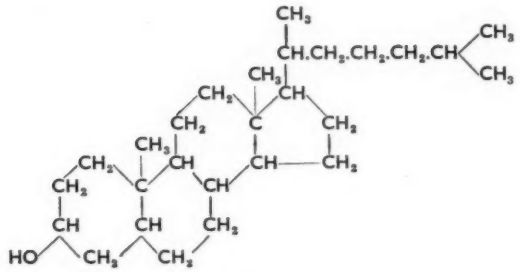
a. Excretory Products

The chemistry of the gonadal hormones came into being as part of the rapid, almost explosive, development of the chemistry of the sterols some 25 years ago. In 1929 two laboratories reported the isolation of the highly oestrogenic compound oestrone, $C_{18}H_{22}O_2$, from the urine of pregnant women (Doisy, Veler & Thayer, 1929; Butenandt, 1929): in 1930 Marrian isolated the slightly less active oestriol, $C_{18}H_{24}O_3$, from the same source (Marrian, 1930a, 1930b). In 1929 pregnanediol, $C_{21}H_{36}O_2$, was isolated (Marrian, 1929). In 1931 the androgen androsterone, $C_{19}H_{30}O_2$, was isolated from the urine of normal males (Butenandt, 1931). Speculation may well have suggested that these compounds were interrelated, but, until development had occurred in what appeared to be another field of organic chemistry, definite clues to constitution were entirely lacking. At this time work on the isolation of vitamin D (calciferol, ergocalciferol) was in progress in England and Germany, and the *x*-ray studies by Bernal (1932a, 1932b) of ergosterol and its irradiation products, which demonstrated the impossibility of the currently accepted formula of the sterols, had provoked Rosenheim & King (1932a, 1932b, 1932c) to propose first the perhydrochrysene and then the perhydro*cyclo*pentenophenanthrene formula (I) for cholesterol. This new theoretical weapon had an immediate clarifying effect on the chemistry of a multitude of compounds, and not least of the sex hormones. Marrian & Haslewood (1932) and Butenandt (1932a) put forward the formulae (II) and (III) for oestrone and oestriol, and Butenandt (1932b) the formulae (IV) and (V) for pregnanediol and androsterone.

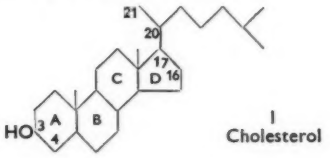
Complete proof of connexion of at least androsterone with the sterols followed from the degradation of *epi*cholestanol (cholestan-3α-ol) to androsterone by Ruzicka, Goldberg, Meyer, Brüngger & Eichenberger (1934), and, moreover, this constitutes the first observation relevant to the stereochemistry of the gonadal hormones.

b. Original Hormones

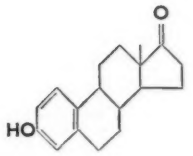
Whilst accessibility and abundance of the source, urine, had allowed the early isolation of the hormonally active compounds



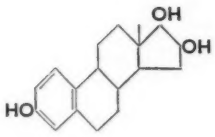
or, in conventionally abbreviated form :



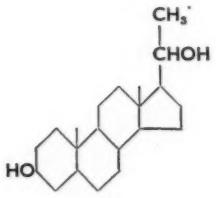
I
Cholesterol



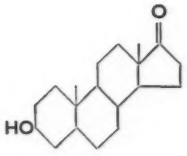
II
Oestrone



III
Oestriol

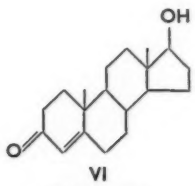


IV
Pregnanediol



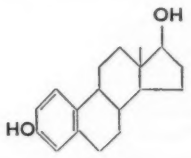
V
Androsterone

oestrone, oestradiol, androsterone and, later, dehydro*epi*androsterone, it was clear that the original hormones produced by the gonads might differ from these excretory products. In fact, in the case of androgenic activity, Gallagher & Koch (1934) and Dingemanse, Freud & Laqueur (1935) found differences in stability and qualitative differences in the action of concentrates from testis and of androsterone. David, Dingemanse, Freud & Laqueur (1935; cf. David, 1935) were finally able to isolate testosterone (VI) from bull testis.



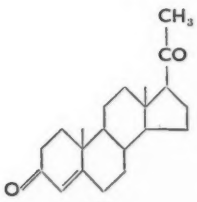
VI
Testosterone

In the case of the oestrogens, the suspicion that oestrone was not the primary hormone arose when it was found that the oestradiol-17β (VII) prepared by reduction was physiologically more potent. In 1935 MacCorquodale, Thayer & Doisy (1935, 1936) succeeded in isolating some 12 mg. of oestradiol-17β from four tons of sow ovaries, accounting for about half of the oestrogenic activity present, and it seems established that this is the primary hormone.



VII
Oestradiol

No indication of the identity of the primary progestational hormone, or progestogen, had been obtained before it was actually isolated from tissue, for pregnanediol was quite inactive biologically. The hormone, progesterone, was isolated in 1934 in four laboratories, and the constitution (VIII) was proposed by Slotta, Ruschig & Fels (1934): it was not until 1937 that pregnanediol was unequivocally recognized as a metabolite of progesterone (Venning & Browne, 1937).



VIII
Progesterone

Henceforward, it might seem, a skeleton of knowledge of the chemistry of the gonadal hormones could be acquired by any medical student, for it could be reduced to textbook terms in a few simple formulae having in common the four-ring steroid nucleus, and this lay figure was decorated with groups in appropriate cases attached to the important 3- or 17-positions, varied occasionally by a double bond between 3 and 4 or substitution at 16, 20 or 21 (cf. formula I). In those early days, from the clinical point of view, it seemed that examination of the excretory products in the urine might be expected to provide an indication, albeit a rough one, of the intrinsic hormone production of the body.

2. Occurrence in the Body

However, this abbreviated story, whilst mostly true in essentials, is only an indicative sketch and far from being a complete picture of the chemistry and the occurrence of gonadal hormones in organ tissues and of degradation products in the excreta. As a result of subsequent work it appeared that certain groupings and configurations are associated with maximal biological activity, the specificity of constitution to activity varying in each type of hormone. Thus oestrogenic activity is possessed by a great variety of compounds; nevertheless oestradiol-17β is very much more active than oestradiol-17α, in which the 17-hydroxyl group is on the other side of the plane of the ring system. Again, androgenic activity is maximal with Δ^4-3-oxo-compounds, e.g. testosterone, and with the 17-substituent in the β-position (above the plane of the paper as the formula is usually written). Few progestogens are known, and the most active has the Δ^4-3-oxo-group and 17β-substituent. The biochemical picture is complicated by the circumstance that hormones occur elsewhere than in the gonads and sometimes in what seem to be the wrong places. The adrenal cortex is found to have, in addition to its own proper family of "corticosteroids" with the characteristics of a two-carbon side-chain at 17, giving a total of 21 carbon atoms and an oxygen atom in the 11-position, also oestrone, progesterone, and androst-4-ene-3:17-dione (IX), together with adrenosterone, androstane-3β:11β-diol and 17α-hydroxyprogesterone, the last four of which have androgenic activity. The placenta, the chemical investigation of which is by no means exhausted, was early found to contain oestrogens, but it was not until three years ago that the theoretically expected hormone, progesterone, was extracted by Pearlman & Cerceo (1952). Ovaries contain androgens (Parkes, 1937, 1950), and physiological evidence of production of androgens is provided by experiments in which ovaries, exteriorized by transplantation to the ears of castrated male mice (Hill, 1937) or rats (Deanesly, 1938), maintained the accessory sexual organs. On the other hand, material with progesterone-like activity can be extracted from testes (Callow & Parkes, 1936). The greatest paradox—and as yet unexplained—is the occurrence of oestrogens in the male, and there must be some peculiar eccentricity of equine biochemistry to account for the circumstances that the tissue richest of all in oestrogens is the testis of the stallion, and that the same animal excretes a higher concentration of oestrogen in its urine than any other, with nearly twice the output a day even of the pregnant mare (Zondek, 1933, 1934a). Further problems are provided by the finding of substances related to the gonadal hormones, both in tissues and in urine, which may possess biological activity, or may be inactive but are obviously so chemically like active compounds that their presence must be of importance.

Work of the last 20 years has been devoted to investigating the genesis, mode of action, and metabolism of the steroid hormones in normal and diseased states in man and other animals, and, in spite of accumulation of detailed results, many questions remain unanswered.

3. Genesis

Least, perhaps, is known about the genesis of the gonadal hormones. The simplest assumption is that they are derived from cholesterol by degradation of the side-chain and modification of ring A. A simple but convincing experiment by Bloch (1945) demonstrated this possibility; he administered deutero-cholesterol to a woman in the eighth month of pregnancy and recovered from her urine such amounts of pregnanediol containing deuterium that one-half to two-thirds must have arisen by degradation of the cholesterol. Presumably the sites of these chemical transformations from cholesterol to gonadal hormones are the organs of origin, but few clues to the mechanism have been obtained. Successful experiments with gonadal organs, analogous to those in

which the adrenal cortex is perfused with labelled intermediates, have yet to be reported, but Rabinowitz & Dowben (1955) have reported the incorporation of [α-^{14}C]acetate into oestradiol and oestrone by tissue slices or pulps of dog ovaries.

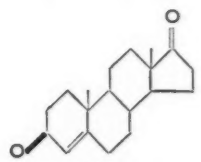
4. Mode of Action

The mode of action of the gonadal hormones is practically unknown. At cellular level recent observations by Huggins and his group (Huggins, Jensen & Cleveland, 1954; Huggins & Jensen, 1954) would seem to be of fundamental importance. Hypophysectomized rats with atrophic uterus and vagina gave readily differentiated histological responses in these tissues to administration of steroids, depending on the number and site of functional groups, the configuration of the molecule and the state of oxidation. As a working hypothesis, the idea, now familiar from enzymology, of active molecules being able to approximate closely to a specific surface, is consistent with the results.

At a " biochemical " level, the relationship between gonadal hormones and enzyme activity has been the subject of a very large number of papers in recent years. These have been reviewed by Fishman (1951) in the field of oestrogens and by Dorfman (1952a) for steroids as a whole. Much of this work has been inspired by the hope that, as with many vitamins, the characteristic action of hormones is to be found in their participation, whether as coenzyme or as substrate, in essential, specific, enzyme systems. Alternatively their activity may be exerted by bringing about change in enzyme concentrations in tissues, by acting as accelerators or inhibitors, or by affecting accelerators or inhibitors. There is as yet no evidence that steroids can function as components of enzyme systems, but there is an impressive list of observations of less direct influence on enzyme activities, though what this may signify is far from clear. Certain steroid hormones control in part the concentration of arginase, amino-acid oxidase, cholinesterase, phosphatases, β-glucuronidase, esterase, proline oxidase, adenosinetriphosphatase, and choline acetylase. In some instances this controlling activity is specific to certain tissues. Certain inhibitory activities of steroids on enzyme systems, D-amino-acid oxidase and the α-glycerophosphate-dehydrogenase system, have been described, but the relation of these activities to those of steroid hormones in the intact organism is, to say the least, questionable. An even less direct action, namely, on inhibitors or accelerators, is not certainly demonstrated, but may account for some of the supposed effects on enzyme concentrations. The significance of all this work would be much clearer if it were known more precisely how the particular enzymes act in the task of maintaining the accessory sexual organs: this is the special and essential function of the gonadal hormones, and it is the mechanism of this which we want to have explained.

5. Degradation in Vitro

Another aspect of metabolism of gonadal hormones is that of the mechanism of destruction or degradation, and a great volume of work has been recorded (cf. the reviews by Pearlman (1948a) and Segaloff (1949) on oestrogens, and by Samuels & West (1952) on other steroid hormones). This work originated in an early observation by Zondek (1934b). He endeavoured to find why it was that such a very large proportion of oestrogenic potency was lost and could not be accounted for when oestrogens were administered to animals. He found that oestrogenic potency was lost rapidly when oestrone was incubated with rat-liver mince. The course of this destruction remains unknown, for no products have been recognized, even in the most recent work by Ryan & Engel (1953a, 1953b), in which the interconversion of oestrone and oestradiol by rat liver or by a variety of human tissues was studied. In studying metabolism of pregnane-3α: 20α-diol by rat liver, Grant (1952) was able to obtain a small yield of pregnan-3α-ol-20-one by an enzyme system requiring diphosphopyridine nucleotide, but 90% of the material was lost as unrecognized products. Conversion of pregn-5-en-3β-ol-20-one to progesterone in a yield of 12% by a pulp of human placenta has been observed by Pearlman, Cerceo & Thomas (1954). The study of the metabolism of androgens by tissue in vitro has, in a sense, been more productive, for it has been possible to detect and separate products of dehydrogenation and hydrogenation in fair yields: this work has been carried on in an atmosphere of controversy. Against some work the objection has been made that conditions of reaction were grossly unphysiological when the steroids were in suspension or were added as water-soluble derivatives. Unfortunately, if this criticism is to be met, the investigations require even more complicated and time-consuming techniques, and even then the results may be said to represent what can happen in vitro but to give no indication of what does happen in the body. Fishman (1949) went so far as to question whether in-vitro work meant anything. " True ", he said, " a homogenate of liver will destroy estrogen and androgen but do we have homogenized liver in our bodies ? " On the other hand, there is clinical evidence that, in the case of oestrogens at least, damage to the liver may entail over-action of hormones in the body. Even if the action of the liver is purely a mechanism for disposing of excess, its possible activity merits investigation. The picture which emerges from work currently reported is of the presence in liver of a cytoplasmic dehydrogenase—which can be concentrated by precipitation with ammonium sulphate (Sweat, Samuels & Lumry, 1950)—requiring diphosphopyridine nucleotide and oxidizing the 17-hydroxyl of testosterone to a 17-oxo-group, and of less clearly defined enzyme systems capable of reducing the ketone and ethylenic bond in ring A (see formula VI). It has been debated whether androst-4-ene-3: 17-dione (IX) accumulates, as Kochakian and his school maintain, or, as Samuels and his school find, does not accumulate even under conditions designed to give a maximum yield of 17-ketones. Work done in my laboratory by P. Ofner (unpublished) with liver mince and true solutions of testosterone, which was labelled with ^{14}C in some experiments, seems to demonstrate, under conditions of complete destruction of testosterone and of maximal 17-ketone formation, that androst-4-ene-3: 17-dione is a product that can be isolated in at least 20% yield,



IX
Androstenedione

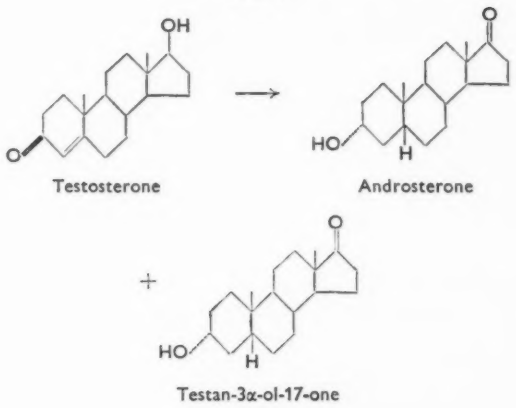
accompanied by more polar products in larger amount. An important controversial point is whether, as Samuels maintains, the liver is the principal site of degradation, or, as Wotiz and his co-workers state (cf. Wotiz, Lemon & Voulgaropoulos, 1954), a subsidiary site, and exceeded in activity, at least in man, by other tissues, whilst even serum is active in presence of diphosphopyridine nucleotide (Richterich-van Baerle, Wotiz & Lemon, 1954).

6. Metabolism in Vivo

Hardly a link has been found to connect these studies with the much more practical, clinically important results on the total, over-all metabolism of gonadal hormones which has been studied since the early days of hormone isolation. It is now established that in man the route of excretion of steroid hormone metabolites is in the urine—unlike rodents, in which faecal excretion occurs. In the urine hydroxy-compounds occur as ethereal sulphates or glucosuronides (glucuronides in older terminology), the site of formation of which is undetermined. For isolation or estimation, hydrolysis is necessary, and in the course of this vigorous process, which entails boiling with mineral acid, some destruction of the original compounds and some formation of artifacts occurs. However, with the limitations imposed by these circumstances, a considerable amount of useful information has been accumulated on the fate of endogenous and exogenous gonadal hormones in the human organism—in fact, so much that the present account can only be highly compressed and selective, and the reader is referred to fuller accounts given, for example, by Engstrom (1948), Dorfman (1948, 1952b, 1952c), Dorfman, Ungar, Vignos, Stecher & Shumway (1952), Dorfman & Ungar (1953), Pearlman (1948a, 1948b, 1952), Heard & Saffran (1949), and Dobriner & Lieberman (1952).

Work on the metabolism of gonadal hormones began with the androgens and attempts to demonstrate to the biologist and the clinician that a chemical indicator of the amount of a particular type of steroid was not only more practical, but a more reliable guide than biological activity to the excretory products derived from the original hormone. This entailed the development of Zimmermann's reaction and its adaptation to rapid and sensitive determination of 17-ketosteroids in urine extracts (Callow, Callow & Emmens, 1938; Medical Research Council, 1951). It was found that the amounts of urinary androgen in men, measured biologically or chemically, gave values which, although somewhat greater on the average than those in women, varied over a range which overlapped the range of variation of normal women. This observation led to closer chemical examination, which showed that urine from both sexes contained androsterone, androst-5-en-3β-ol-17-one (dehydro*epi*androsterone) and testan-3α-ol-17-one (aetiocholan-3α-ol-17-one) (Callow & Callow, 1939). Since androsterone and testan-3α-ol-17-one had been identified by N. H. Callow (1939) as transformation products (fig. 1) of testosterone administered to a eunuch, but these same compounds also occurred, albeit in small amount, in the urine of a eunuch receiving no hormone therapy (Callow & Callow, 1940) and of ovariectomized women (Hirschmann, 1939), the specificity of androgen excretion to any one source, whether hormone or gland, could not be upheld; it could only be assumed that secretion by the adrenal cortex was the source of some at least of these compounds. The war put an end to investigations in my laboratory, but other

FIG. 1



laboratories continued to pursue the problems of androgen metabolism, and the outstanding work of Dorfman in particular may be singled out for mention. In this work the theoretical possibilities of derivation from " key compounds " —it is interesting to note that androst-4-ene-3:17-dione (IX) is one of these—have been explored and checked with the steroids found in normal urine and in urine after administration of supposed intermediates.

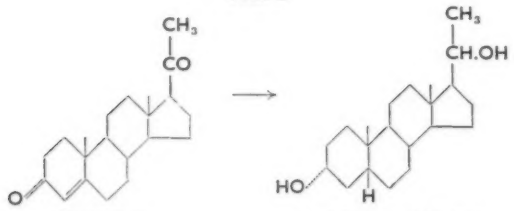
The practical use of examination of urine for androgens and related compounds is in diagnosis of certain types of tumour; thus some adrenal cortical tumours show their presence by greatly increased excretion of dehydro*epi*androsterone, and observation of this can be an important diagnostic aid (cf. Crooke & R. K. Callow, 1939; N. H. Callow & Crooke, 1944). More detailed fractionation may be of value in certain special cases (cf. the discussion by Pond, 1954). The case of an interstitial cell tumour of the testis with metastases in the liver is worthy of mention because of the " record " level of excretion of androsterone as the sulphate and the indication it gives that the source of androgens in the testis is the interstitial tissue. The tremendous effort of Dobriner and his colleagues (for references, see Dobriner & Lieberman, 1952) in the correlation of hormone metabolites and precursors, and in determining the changes in the pattern of excretion that follow glandular changes or disease, must at least be mentioned, but further discussion would be beyond the scope of this article.

The study of oestrogen metabolism and excretion was for long hampered by difficulties in separating the minute amounts of highly active material, but advances in fluorimetric methods of analysis and in methods of separation (Engel, Slaunwhite, Carter, Ekman, Olmsted & Nathanson, 1952; cf. Engel, Slaunwhite, Carter, Olmsted & Nathanson, 1952) have altered the position to some extent. The fact that a new natural oestrogen—" 16-*epi*oestriol " or oestra-1:3:5-triene-3:16β:17β-triol—has only recently been isolated from urine of pregnancy (Marrian & Bauld, 1955) and that yet more new compounds in this class are reported (Stern & Braunsberg, 1955) is sufficient indication that work in this field is still in a pioneering stage.

The metabolism of progesterone, in spite of having been under investigation for some 18 years, and in spite of the apparent simplicity of the transformation of progesterone to

pregnane-3α : 20α-diol (fig. 2) is still little understood. In spite of some reports of practical methods of assay for clinical purposes, there are acute difficulties in the accurate assay of pregnanediol in urine, and metabolites from the adrenal cortical hormones, with the same two-carbon side-chain, have to be distinguished from those of the corpus luteum.

FIG. 2



Progesterone — Pregnane-3α : 20α-diol

It is hardly possible adequately to discuss the metabolism of gonadal hormones in isolation from the adrenal cortical hormones, but it is clearly inexpedient to extend this discussion outside the scope indicated by the title. What can be stated with confidence is that the therapeutic use of cortisone and the associated recent developments in the synthetic chemistry of adrenal cortical hormones, new micro-techniques, and the experimental approaches these have made possible, have produced a new wave of energetic attack on an allied front which includes the gonadal hormones, and that with this impetus answers to some of the steroid metabolic problems discussed here may well be obtained.

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SYNTHETIC OESTROGENS

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1 Discovery and development
2 Biological activity
3 Uses of synthetic oestrogens
4 Synthetic oestrogens and cancer
References

Today it is necessary to distinguish between synthetic oestrogens and artificial oestrogens. Prior to the total synthesis of oestrone by Miescher and his colleagues (Anner & Miescher, 1948), the only synthetic oestrogens were the analogues or artificial ones, but today the term could apply to both the naturally occurring and to the latter. The present article will be restricted to the artificial oestrogens or hormone analogues.

1. Discovery and Development

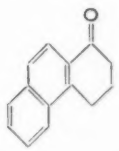
The first announcement of the discovery of a synthetic oestrogen was made in the year 1933 by the present writer in conjunction with Cook and Hewett (Cook, Dodds & Hewett, 1933). We were led to experiment with various compounds similar in structure to the oestrogenic hormones, because of the fact that at least five naturally occurring substances possessed oestrogenic activity. It must be remembered that at this period hormone specificity was regarded as being very exact. There was, for example, only one adrenaline in the animal kingdom, only one thyroxine, only one insulin, and so on. The general view of physiologists and pharmacologists was that the hormone locks were extremely specific and that only one key would open them. Doubt was first cast on this in the late 1920's and early 1930's when it was found that the urine of pregnancy of different animals contained oestrogens which differed slightly from each other. For example, the urine of human pregnancy contains mainly oestriol whereas that of equine pregnancy contains oestrone. By the early 1930's at least five substances had been demonstrated in nature to possess oestrogenic activity. These were: oestradiol, oestrone, oestriol,[1] equilin and equilenin.

This was the first occasion when a variety of substances of natural origin were all shown to be capable of producing the same hormonal or physiological effect. It occurred to us that the lock represented by oestrogenic activity might be not quite so specific as the other hormonal activities and we therefore decided to investigate how far it was possible to depart from the structure of the natural hormone and still obtain activity.

[1] See Callow, p. 126 of this number of the Bulletin.—ED.

Since all the substances contained the phenanthrene-ring system, we decided to experiment in the first instances with the compounds with this basic constitution. After a long series of experiments we were able to show that activity was possessed by a relatively simple phenanthrene compound, namely, 1-oxo-1:2:3:4-tetrahydrophenanthrene. This is shown in fig. 1.

FIG. 1



1-oxo-1:2:3:4-tetrahydrophenanthrene

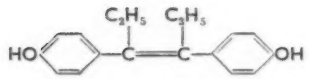
It must at the outset be pointed out that this compound, whilst qualitatively similar in activity to oestrone, quantitatively is very much weaker. It requires 100 mg. of this substance to produce the same effect as 1 μg. of the naturally occurring substance. The importance of this observation, however, is that the lock can be picked by a skeleton key which bears some—but slight—resemblance to the naturally occurring product. We were then able to show that various dibenzanthracenediols had similar activity.

It is not proposed to go in detail over all the steps leading to the final discovery of the highly potent stilbene derivatives which are now used extensively in clinical medicine. Sufficient to say that we (Dodds & Lawson, 1936) were able to show that the phenanthrene-ring system was not necessary and that relatively simple carbinols could possess oestrogenic activity. From this it was shown that stilbene itself was relatively active but that 4:4′-dihydroxystilbene possessed even greater activities. On theoretical grounds the activity of *p*-hydroxypropenylbenzene, or anol, was studied and was reported (Dodds & Lawson, 1937a, 1937b) to have roughly the activity of oestrone. Re-examination of this work showed that only certain samples of the substance possessed activity, and it was concluded that any potency was due to a contaminant. Again, on theoretical grounds, it was most likely that this was a polymer, since substances of the type of anol undergo polymerization very readily; on speculative grounds the most likely active contaminant was the symmetrical dimeride of anol, which is a substance now known as stilboestrol.

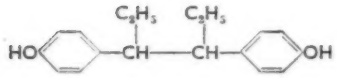
Sir Robert Robinson and his colleagues at Oxford had been working on similar problems, and, as a result of a combination of our resources, the highly active dimeride of anol, diethylstilboestrol, was produced fairly rapidly (Dodds, Goldberg, Lawson & Robinson, 1938). The reason for the use of the term diethylstilboestrol was that there might be other more active substances in this series. We regarded 4:4′-dihydroxystilbene as the basic, or parent, substance; this we termed " stilboestrol ", and we then added the substituents on the αβ-carbon atoms, such as diethyl. A long investigation showed us that maximum activity rested with the symmetrical diethyl compound, and in subsequent practice the word diethyl has been dropped and the substance is generally known as stilboestrol.

Closely related in this series are two other substances, hexoestrol and dienoestrol (fig. 2). Hexoestrol represents the

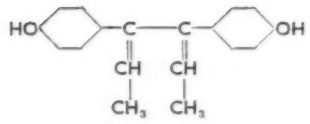
FIG. 2



Stilboestrol



Hexoestrol

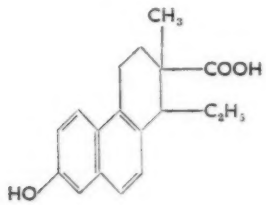


Dienoestrol

hydrogenated variety of stilboestrol in which the double bond between the two central carbon atoms has been reduced. This hydrogenation produces two asymmetric carbon atoms, with the result that isomers are possible. The most active compound, the one used in clinical practice, is the internally compensated or *meso* form. In the case of dienoestrol we have a double bond in the side-chain. Practically all the clinical work that has been done with synthetic oestrogens has been made with these three substances. There are, however, many other synthetic oestrogens. It will be necessary to review these very briefly.

Miescher and his colleagues (see Miescher, 1944) have shown that if the five-membered ring of oestrone be opened, the acids of the corresponding phenanthrene compound possess considerable oestrogenic activity. These he has referred to as doisynolic acid, to commemorate the name of E. A. Doisy, a pioneer in this field. The most active in the series is bisdehydrodoisynolic acid; its formula is given in fig. 3.

FIG. 3



Bisdehydrodoisynolic acid

This substance is difficult to manufacture, and, whilst undoubtedly of high activity, is not commonly used because of the relative ease of the manufacture of stilboestrol and similar substances.

Proceeding on the disintegration theory of the *cyclo*pentenophenanthrene-ring system, Horeau & Jacques (1947) produced a series of compounds which represented derivatives obtained by opening ring C of the doisynolic acid series. This gave acids derived from naphthalene, of which allenolic acid is the substance which has received most attention. This has been used as an oestrogen for clinical purposes in France and is undoubtedly highly active. Again it has not found the extensive application that the more simply manufactured stilbene derivatives enjoy.

Robson & Schönberg (1937) have shown that triphenylethylene possesses oestrogenic activity, and also that certain halogen derivatives are active. Whilst these substances do not possess the high activity per unit weight of the aforementioned oestrogens, they have the property of very prolonged action. This is probably owing to their solubility in the fat depots of the body.

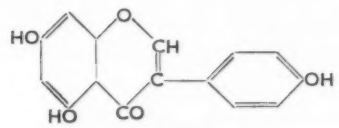
All the above-mentioned substances are purely synthetic and are in no way derived from a naturally occurring substance. It is, however, in this review necessary to consider another group of which there is only one example at present, that is, chemical alterations of the naturally occurring substances. It is interesting to note that, when oestrone was isolated very early in the 1930's, it was shown that it possessed a ketone group in the 17-position. Furthermore, it was demonstrated that, if this ketone be reduced to a secondary alcohol, a substance of greatly enhanced activity was produced. This possessed two hydroxyl groups, one in the 17-position and one in the 3-position, and was known as oestradiol. Some years later the substance was obtained naturally from follicular fluid; this is one of the few examples of a substance's being produced in the laboratory from a naturally occurring compound of greater activity than that known at the time, and subsequently to be found as a natural product. It has been shown that it is possible to introduce an ethynyl substituent in the 17-position of oestradiol, and that this substance, ethynyloestradiol, possesses a very great activity when given by mouth.

Space does not permit us to review and list all the recorded synthetic oestrogens.[1] As an example, may be cited the series of substances possessing oestrogenic activity attained by increasing the aliphatic block between the two benzene rings from two to three carbon atoms—this gives a potent oestrogen known as benzoestrol. Simple phenols have been shown to possess low, but undoubted, oestrogenic activity (Lawson, 1954). A new series of oestrogens has recently been brought to light by interesting work in the veterinary field in Australia:

> In Australia (Bennetts & Underwood [1951]) it has been known for some considerable time that certain pastures led to a diminution in the fertility of sheep and the defect has been shown to be in the ewes, the potency of the rams remaining quite normal. Histological examination of the uterine mucosa showed a condition of hyperplasia very similar to that occurring under the influence of heavy oestrogenic administration. It was also known that these pastures were overgrown with subterranean clover, a very rich form of feeding stuff and it was only natural that an investigation should be made into the possibility of this constituent in the pasture supplying the oestrogens. Extracts were made of subterranean clover and it was very clearly shown that an oestrogen could be extracted from this material. This substance was capable of producing oestrus when injected into ovariectomised rats or mice in exactly the same way as the naturally

[1] See review of synthetic oestrogens by Solmssen (1945).

occurring oestrogens. Extensive work has been conducted on the active constituent of subterranean clover. There is considerable evidence (Bradbury & White [1951]) that at least one oestrogenic substance is the flavone genistein . . .



Genistein

. . . Various derivatives of genistein have been found and in woods similar types of compounds are encountered in considerable quantity.

These interesting observations from Australia stimulate reflections that there may be possible precursors of stilboestrol like substances in nature.

The foregoing summary (Dodds, 1953) gives a fairly representative picture of the various classes of oestrogenic substances. It is perhaps safe to say that in no other field is there such a variety of substances possessing the same biological activity. Many attempts have been made to try to find some common denominator in this vast variety of heterogeneous organic compounds. It must be admitted, however, that up to the present no theory of activity would explain the whole series. Obviously much more work will have to be done on the basic principles of the oestrogenic response before we are able to offer an explanation.

2. Biological Activity

The synthesis of stilboestrol was first published in 1938, and since that time a vast literature has grown as to its use both clinically and experimentally. Hexoestrol and dienoestrol have been extensively used clinically, but not to the same extent experimentally. Ethinyloestradiol has found wide acceptance as an orally active oestrogen for clinical purposes, whilst the other substances, namely the doisynolic acid series and the allenolic acid series, have not been so popular. It is, therefore, proposed to confine this review to biological and clinical activities of the stilboestrol series.

In general terms it may be stated that the artificial oestrogens possess all the activities of the naturally occurring ones. These are vaginal cornification, and development of puberty and of accessory organs, such as opening of the vagina in rodents, enlargement of the breasts and the characteristic uterine changes. One of the most important biological actions of oestrogens is their power, when given in large enough doses, to inhibit the functional activity of the anterior lobe of the pituitary. After the administration of oestrogens there is an increase in the size of the pituitary, associated with characteristic alterations in the staining of the cells. At the same time the production of certain of the trophic hormones is interfered with. Thus the growth hormone and the gonadotrophic hormones cease to be produced, with the result that if the animal is immature it fails to grow, whereas if it is mature there is involution of the accessory organs, and results resembling those of castration are obtained.

The stilboestrol series differ markedly from the naturally occurring hormones in that they are highly active when given by mouth. It is this latter property which has been largely responsible for the great increase in the use of oestrogenic agents. Prior to the discovery of stilboestrol, the clinician had to inject the hormone, usually in the form of benzoates, and this naturally limited its clinical use. The same also applied to the veterinary field. In stilboestrol we have a very cheap, highly active substance when administered by mouth and, therefore, the drug can be used freely in the out-patient clinic and also in veterinary practice.

3. Uses of Synthetic Oestrogens

As already pointed out, by far the greater experience has been had with stilboestrol and, whilst the other synthetic oestrogens would doubtlessly be equally useful, their production presents greater trouble and expense.

In clinical work on man stilboestrol has been used in all the conditions which require the employment of oestradiol in its various forms; in gynaecology its greatest use has been in the treatment of delayed puberty, the suppression of lactation on weaning, and the treatment of the menopause. In the last mentioned, it has been highly successful; as little as 0.1 mg. three times a day will control most of the symptoms, particularly the very distressing vasomotor phenomenon known as "flushing". In the early days marked toxic reactions were frequently noted. These consisted of nausea, vomiting and—in some cases—marked vertigo. With the reduction of doses these symptoms ceased to be a problem, although occasionally some patients who show these side-reactions are encountered. Usually these pass off if the use of the drug is persisted in. We have no knowledge as to the actual underlying cause of these reactions, but it may well be that the drug is more stable than the naturally occurring substance and that, therefore, it persists longer and produces nausea in exactly the same way as the flooding of the body by oestrogen in early pregnancy produces vomiting of pregnancy. Clinicians have maintained that there is a marked difference in the liability to produce side-effects between the three oestrogens, stilboestrol, dienoestrol and hexoestrol, it being claimed that hexoestrol, weight for weight, is the least likely to cause adverse symptoms. Bishop's researches have given us a very clear explanation of this interesting observation. Using as a criterion the bleeding caused by oestrogen withdrawal, Bishop (1954) was able to place the three substances in their order of potency and to show that this was quite different from that obtained by a study on animals of the reaction of the substances. He found stilboestrol the most potent, dienoestrol coming second with roughly 90% of the activity of stilboestrol, whilst hexoestrol came a very poor third with an activity of about 10%. We can, therefore, see that if one gives the same dose of hexoestrol as stilboestrol, one is giving only one-tenth of the oestrogenic activity. When the other two, namely dienoestrol and hexoestrol, are given in the same oestrogenic potency as stilboestrol, it is said that the incidence of side-reactions is the same in all three.

Some workers have claimed that it is possible to demonstrate selectivity in the various aspects of oestrogen activity. They claim, for instance, that it is possible to produce substances which will have a powerful inhibitory action on the anterior lobe of the pituitary and yet be without action on the vaginal cornification reaction. This matter has been the subject of the most careful investigations in the Courtauld Institute of Biochemistry, and we have nearly succeeded in showing the possibility of dissecting the action of these

various substances; in our experience it is not possible to find an oestrogen that will inhibit the anterior lobe of the pituitary and yet will have little action peripherally.

4. Synthetic Oestrogens and Cancer

The use of stilboestrol by Huggins and his collaborators (Huggins & Hodges, 1941) to treat carcinoma of the prostate is now well known and established. It is generally assumed that this beneficial action is due to a castration effect, caused by inhibiting the functional activity of the anterior lobe of the pituitary and thereby stopping the production of gonadotrophic hormone. Extensive trials with stilboestrol in treatment of carcinoma in sites other than the prostate have been undertaken, and out of the wealth of publications on this subject it would appear that it is only in inoperable carcinoma of the breast that the use of stilboestrol is justified. Competent authorities maintain that, in some 20% of cases, beneficial results are obtained. These are shown by a shrinkage of the original growth and by diminution in size of secondary deposits. Whilst this finding is of the greatest theoretical importance, it cannot be too strongly emphasized that synthetic oestrogens are in no sense a cure for any form of malignant disease.

* * *

It would be impossible to attempt to summarize the various uses to which stilboestrol has been put. These vary from its administration to prisoners with criminal sexual drive, to the implantation of pellets in poultry in order to produce physiological capons. As for stilboestrol as a biological tool, there is again a vast literature on the results obtained by inducing a strong oestrogenic background by the administration of the substance.

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BIOLOGICAL ASSAY OF THE GONADAL AND GONADOTROPHIC HORMONES

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4 Relaxin
5 Gonadotrophins
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Biological assay is used for a variety of purposes. At best, it is a reasonably precise method of standardization, as in the control of insulin manufacture. At worst, yet often at its most essential level as a research tool, it is a very rough-and-ready guide to the amount of something present, the exact nature and properties of which we do not even know.

In this article, it is not proposed to deal with statistical or indeed much technical detail of any description. The requirements and characteristics of satisfactory assays have been discussed at length by Gaddum (1931), Bliss (1941), Emmens (1948), Finney (1952)[1], and many others. Standardization, as in the control of production of therapeutic substances, has been reviewed fairly recently by Emmens (1951). Attention is focused here mainly on research and clinical requirements, and thus on the assay, although not the preparation, of such material as blood and urine extracts. Even so, much is necessarily omitted.

As chemical or physical methods of assay become practicable, the bio-assay of any particular substance should cease, except perhaps when it is tedious or impossible to purify a compound to a sufficient degree for other than biological assay, or when the latter method is much more sensitive. Although many steroids can be characterized chemically in very small amounts, for these reasons their biological assay continues. However, the estimation of androgenic activity has largely been replaced by 17-ketosteroid determinations; while that of progestational activity has never really got under way, and has been substituted in clinical practice by pregnanediol estimation. Biological assay of oestrogens is so very much more sensitive than any other method that it is likely to continue for some time.

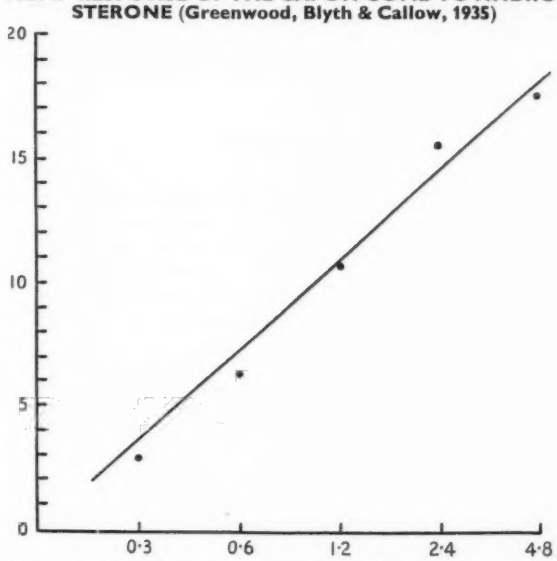
1. Androgens

For biological assay, the international unit (i.u.) of androgenic activity is that of 0.1 mg. of international standard androsterone.[2] Testosterone, the most active naturally occurring androgen, although present in the body, is little excreted in the urine, and metabolites such as androsterone and its stereoisomers are found instead. Some of these are biologically active, and so assay of extracts of urine gives a reflection, but only a reflection, of androgen production in the body, affected in various and mostly little-known ways by other endocrine and metabolic factors. Some of the steroids in urine come from the adrenals, so that a biological measurement of urinary androgenic activity, no less than a chemical assay of 17-ketosteroids or other steroids, gives a mixed assessment.

Growth of the atrophic comb of the capon was first used as a method for the detection of male hormone, and adapted as an assay procedure by Gallagher & Kock (1935) and Greenwood, Blyth & Callow (1935). Other detailed studies by McCullagh & Cuyler (1939) and Emmens (1939a) followed. In these tests, the birds are injected daily for three to five days with oily solutions of the androgen, and comb growth is measured on the fourth to sixth day. The increase in growth is expressed in various ways, a popular method being to measure the length of the comb and the height of a particular spike and to add the increases together. When this index is related graphically to dose (Emmens, 1939a) or log dose (Greenwood *et al.* 1935) a straight line may result, as seen in fig. 1. Such an assay requires 1–2 mg. of androsterone in total for each bird, and thus might demand 30 mg. or 40 mg. of androsterone or its equivalent, or the extract from several litres of urine, for a single determination.

Much greater sensitivity is obtained with inunction of an oily solution directly to the comb, when a useful response is obtained to 2–4 μg. of androsterone, an increase of some 500-fold in sensitivity. Bird for bird, this method of assay is rather less precise, but very much less total urinary or other

FIG. 1. RESPONSE OF THE CAPON COMB TO ANDROSTERONE (Greenwood, Blyth & Callow, 1935)



Abscissae: dose of androsterone (mg.) (logarithmic scale)
Ordinates: growth of comb (increase in length + increase in height) (mm.)

Androsterone was injected daily for 5 days; groups of 5 birds were used.

[1] See also Finney, *Brit. med. Bull.* 1951, 7, 292.—Ed.
[2] In 1950 the Expert Committee on Biological Standardization of the World Health Organization recommended that androsterone should cease to be an international standard when the stock was exhausted, since it is a pure substance capable of chemical standardization.—Ed.

material is required. In addition, the main androgens are very much closer in relative potency and so assessment of activity is less dependent on the particular constituents of an extract.

The chick-comb response was first investigated by Ruzicka (1935) and Burrows, Byerly & Evans (1936) and later by many others. Ruzicka used inunction in this test, painting an oily solution on to the comb for several weeks before measuring a response. In later tests the weight of the comb at the end of the test is used almost exclusively as a measure of response. Dorfman (1948) has investigated this assay exhaustively and finds that within the range of 20–160 μg. of testosterone propionate, with 32 chicks for each substance in a typical assay using two groups on the standard and two on the unknown, errors in the determination of potency are lower than ±38%. This is much the same error as would be given in the capon-comb method by inunction, with the same number of birds, but it uses more androgen.

Androgen assay with birds is inconvenient to most workers, and mammalian methods are much preferred. They are about as accurate, but, although more feasible because of the greater availability of rodents in the laboratory, they involve dissection and take more time and more androgen. These methods are reviewed in detail by Dorfman (1950). The modern tendency is towards rapid tests, such as that of Greene & Burrill (1941) in which immature male rats, 20–22 days old, are given a single injection of androgen. Forty-eight hours later, the seminal vesicles are dissected and weighed fresh; the log dose-response relationship is linear over the range 5–50 μg. of testosterone propionate and, with 40 rats in an assay, limits of error ($P = 0.95$) are about 65–160%. Mathieson & Hays (1945) used castrated rats in a similar test and found limits of error of 74–136% as long as adequate statistical control was employed in the assay to sort out differences between times of castration of the rats.

Dorfman (1954) summarizes some newer, but relatively untried, methods using the protein anabolic effects of androgens.

2. Oestrogens

The international unit of oestrogenic activity is that exhibited by 0.1 μg. of international standard oestrone. Its very small size is consequent on the popularity of the Allen-Doisy test for vaginal cornification in the mouse and it approximates to one " mouse unit ", as used by early workers. There is a second international standard, oestradiol benzoate, of which 0.1 μg. also provides a unit intended for the assay of esterified substances.

Biological assay with rodents, particularly mice, is very widely employed because of its sensitivity and specificity; indeed, an oestrogen is almost solely defined in practice by its capacity to cause vaginal cornification in the rat or mouse. The oestrogens in human urine—oestrone, oestradiol and oestriol—some of which are of adrenal origin, are all quite active and are used as a measure of over-all oestrogen production in the body. When intravaginal tests (see below) are used, the activities of the various natural oestrogens are so much alike that the measure is probably a good one.

Following the work of Allen & Doisy (1932), more has probably been written about this type of assay than about that of any other hormone. Recent reviews by Emmens (1950) and Dorfman (1954) may be consulted for detailed references.

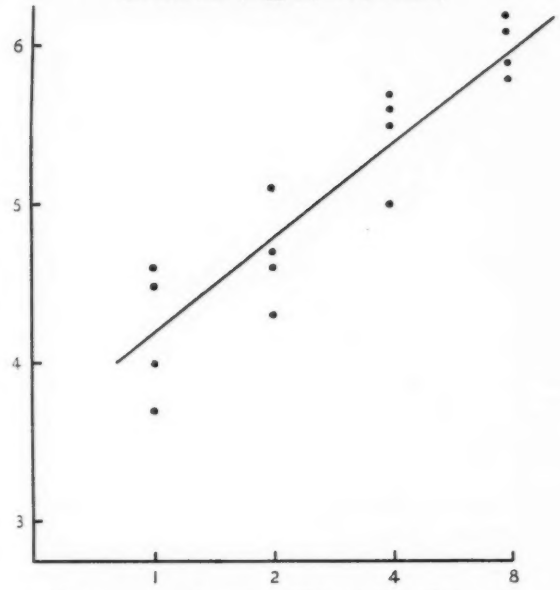
A typical Allen-Doisy test using the subcutaneous route employs 80 ovariectomized mice, each receiving doses ranging around 1 i.u. given in two or more oily or four or more aqueous injections over 36–48 hours. Vaginal smears, taken about 60–84 hours after injection, are scored as positive if they contain no leucocytes and many cornified epithelial cells, and as negative otherwise. Such a test can give limits of error as low as 77–130% at $P = 0.95$.

Detailed investigations of the Allen-Doisy test by Pedersen-Bjergaard (1939) and Emmens (1939a), among others, showed that the relative potency of various natural oestrogens differs enormously according to technique. Thus oestriol has been reported as having a potency which ranges from that of oestrone to one two-hundred-and-fiftieth of this potency, according to different investigators, while Pedersen-Bjergaard found that variations in the assay technique he employed could shift the estimate of potency of a human pregnancy urine extract from 158 i.u./g. to 75,900 i.u./g. It was therefore concluded that the two international standards can be used for comparison with preparations containing only oestrone or oestradiol benzoate; this much limits their application. Despite these drawbacks, the assay of urine extracts continues to be a useful guide.

Intravaginal application of oestrogen in the Allen-Doisy test was tried by Berger (1935) and Lyons & Templeton (1936). They showed that smaller quantities of oestrogen in oily solution than are needed by other routes gave positive responses, but it was not until Mühlbock (1940) used 50% (v/v) aqueous glycerol in mice that very sensitive and consistent results were obtained. The median effective doses (ED_{50}) of oestrone, oestradiol and oestriol were reported by Mühlbock as about 0.00025 μg., 0.0005 μg. and 0.00075 μg. respectively—much the same levelling of potency as with the androgens by local application to the comb, and far greater sensitivity. Robson & Adler (1940) next showed that the natural oestrogens act locally without significant absorption from the vagina into the blood stream when given in threshold doses, and that an ester, oestriol glucuronide, was locally active. Emmens (1941, 1942) showed in a series of studies that nearly all natural oestrogens and some synthetics are locally active, that esterification has almost no effect on activity, and that the median effective doses of all the most potent oestrogens examined are very similar intravaginally. Biggers (1951) has since shown that oestradiol is slightly but significantly more active than oestriol or oestrone, which are of equal local potency. A typical dose-response line for intravaginally administered oestrone is shown in fig. 2.

It has also been shown that oestrogens may be assayed intravaginally in combination with proteins, and that the slope of the dose-response line may be increased, and thus the precision also increased, by several different techniques (Biggers, 1951, 1953; Biggers & Claringbold, 1953; Biggers, Claringbold & Emmens, 1954). In combination with protein, oestrone has an ED_{50} of about one-third of that seen otherwise. Following Albrieux (1941), several investigators have, in effect, " assayed " oestrogens with protein in pellets of dried blood or serum placed intravaginally, or by instillations of whole blood, and have reported positive results, but no systematic studies have come to hand. The very high sensitivity claimed by Sulman (1952) for the intravaginal test seems to be based mainly on an unusual definition of response, which is scored as positive if the number of leucocytes in a smear is less than the number of epithelial cells.

FIG. 2. RESPONSE OF OVARIECTOMIZED MICE TO OESTRONE (Biggers, 1953, Table I)



Abscissae: dose of oestrone (10^{-4} μg.) (logarithmic scale)
Ordinates: response (in probits*)

Oestrone was administered intravaginally; groups of 15–20 mice were used.

* The probit is the normal equivalent deviation plus 5.—ED.

In passing, it should be noted that not all oestrogens can be compared usefully by the intravaginal technique, since the group of pro-oestrogens, as defined by Emmens (1941), has very low local activity in comparison with the rest.

The next most frequent method of oestrogen assay, although far less commonly used than vaginal cornification, is an increase in the uterine weight in rats and mice. Bülbring & Burn (1935) described an eight-day test, and various other authors have given variants, culminating for speed in the six-hour test of Astwood (1938), which operates on a rather different principle (fig. 3). Long-term tests are based on myometrial and other weight increases, Astwood's test depends on a transient change in water content.

In uterine assays, oestriol is usually found to be much more active in relation to oestrone or oestradiol than by injection techniques using other end-points. It also gives a different " ceiling " weight, and so is not strictly assayable in terms of other oestrogens. Otherwise, these assays are reasonably precise: with 40 animals in each test, limits of error ($P = 0.95$) of about 80–125% are feasible, although the promising test of Astwood has never been critically elaborated as a full assay.

When relatively large quantities of hormone are available, the chick-oviduct method is gaining favour, since it is easy to perform and inexpensive (cf. Dorfman, 1954). It has much the same precision as uterine-weight techniques. Uterine or oviducal changes are not, however, nearly as specific as vaginal cornification, and can be caused by other than oestrogenic steroids. The presence of normal quantities of androgen and oestrogen in crude unfractionated urine extracts will not interfere with the estimation of one another when capon-comb tests or vaginal-smear tests are used (Emmens & Bradshaw, 1939), but they might do so in uterine-weight tests.

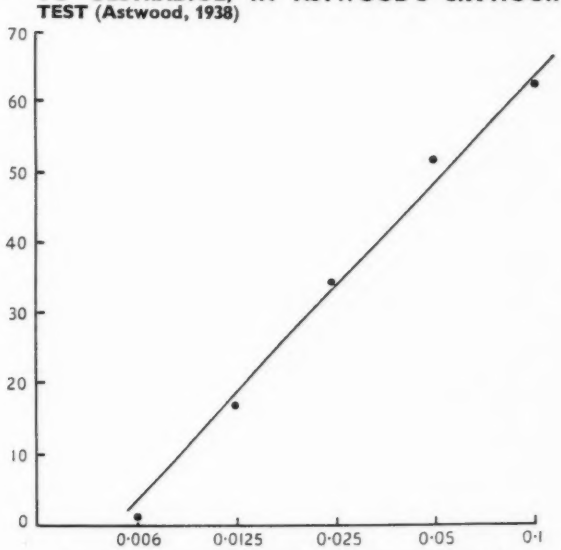
3. Progestogens

Of the naturally occurring steroids, only progesterone has full progestational activity, and very few other compounds show any at all. Ethynyl testosterone (Δ^4-androstene-17(β)-ethynyl-17(α)-ol-3-one) is the most potent of the very few active synthetics, and has some activity by mouth.

Biological assay of progestogens is in a rudimentary stage. The Corner & Allen (1929) test depends on full uterine proliferation in the adult rabbit and was never developed as an assay, while McPhail's (1934) modification using oestrogen-primed immature rabbits went little further. However, a scale of uterine proliferation was given by which rough, quantitative estimates could be made. These tests take up to 1–2 mg. (1–2 i.u.) of progesterone for each rabbit.

Mussio-Fournier, Albrieux, Monato & Gross (1938) found local responses in rabbit uterine segments injected with 0.1 mg. of progesterone, a technique which has been followed in various laboratories. Hooker (1940, 1945) found that, in the mouse, hypertrophy of the stromal nuclei is a specific response to locally administered progesterone, of which as little as 0.0002 μg. is required. Adult female mice are ovariectomized and used 16 days later, when a small amount of progesterone (or test) solution is placed in a ligated uterine segment and the effect assessed two days later. This test, clearly of great value if consistent and specific, has not been extensively used nor investigated statistically, and false negatives have been reported in the presence of oestradiol (Salhanick, Olsen & Hisaw, 1951).

FIG. 3. RESPONSE OF THE IMMATURE RAT UTERUS TO OESTRADIOL, IN ASTWOOD'S SIX-HOUR TEST (Astwood, 1938)



Abscissae: dose of oestradiol (μg.) (logarithmic scale)
Ordinates: increase in uterine weight (%)
300 rats were used.

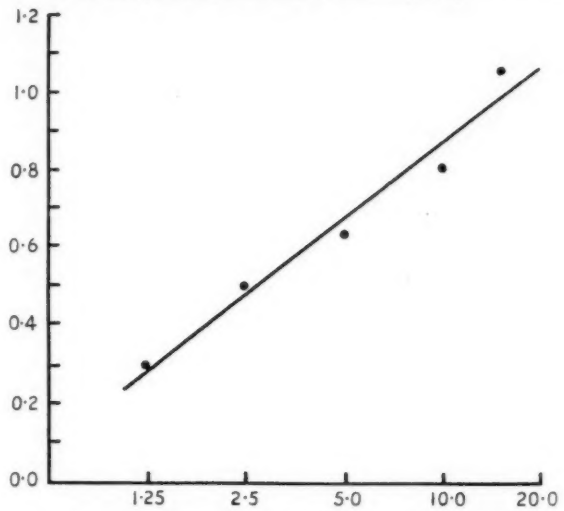
4. Relaxin

Relaxin was first named by Hisaw (1926) and for some time its existence was doubted, since a mixture of oestrogen and progesterone can cause relaxation of the symphysis pubis. Abramowitz, Money, Zarrow, Talmage, Kleinholz & Hisaw (1944) have shown that 500 times the dose of relaxin needed to cause a response in the guinea-pig is without oestrogenic or progestational action, while Hisaw, Zarrow, Money, Talmage & Abramowitz (1944) give evidence that progesterone acts by stimulating the release of relaxin itself. Relaxin is a non-steroid of unknown constitution, coming from the corpus luteum.

Assay of relaxin has been attempted by measurements of pelvic relaxation in guinea-pigs (Fevold, Hisaw & Meyer, 1930; Abramowitz *et al.* 1944; Talmage, 1947; Talmage & Hurst, 1950) or mice (Hall & Newton, 1946a, 1946b). The mouse test of Hall and Newton depends on *x*-ray photographs of the pubic bones, from which their separation can be measured.

Talmage & Hurst (1950) find an exponential relationship between dose and response from 0.005 ml. to 0.03 ml. of their stock solution, using the increase in pubic bone separation in the guinea-pig, also photographed by *x* rays. In a typical series of tests using 36 observations each, the fiducial limits of error ($P = 0.95$) were stated as being up to $\pm 48\%$. Dorfman (1953) and Kliman, Salhanick & Zarrow (1953) have developed the mouse assay to a degree which allows the performance of a reasonably precise test. A typical dose-response line from the latter authors' work is shown in fig. 4.

FIG. 4. RESPONSE OF THE PUBIC SYMPHYSIS OF THE MOUSE TO AN EXTRACT OF PREGNANT SOW'S OVARIES (Kliman, Salhanick & Zarrow, 1953)



Abscissae: dose of extract (mg.) (logarithmic scale)
Ordinates: increase in pubic gap (mm.)
Groups of 4–10 mice were used.

5. Gonadotrophins

Hypophyseal gonadotrophins are probably three in number —follicle-stimulating hormone (FSH), luteinizing hormone (LH), and luteotrophin, the latter being probably identical in some species with prolactin.

A mixture of FSH and LH is produced by the pituitary gland, and this mixture, short of prior chemical separation, cannot be satisfactorily assayed for its components. Serum gonadotrophin from pregnant mares (PMS) is apparently constant in composition, and has most of the biological properties of the pituitary gonadotrophin mixture, with an emphasis on the FSH component. The inconstancy of these mixtures from the pituitary makes them much less easy to handle than PMS. Chorionic gonadotrophin from human urine has no follicle-stimulating effect in hypophysectomized animals and no action on seminiferous tubules; instead, it resembles LH in most of its actions. In man, however, it seems to have the additional properties of a luteotrophin. Chorionic gonadotrophin is thus relatively easy to assay, being, like PMS, a substance of relatively constant properties.

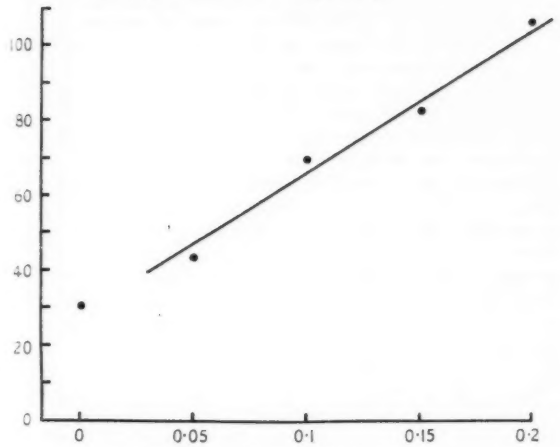
There are international standards for PMS and chorionic gonadotrophin, but these are intended for use only with like substances, and the assay of pituitary gonadotrophins, against these standards, is not strictly permissible. Some tests, such as those depending on secondary responses, of which uterine weight is an example, do not differentiate between FSH and LH and may be used for a rough, over-all assessment. Other tests, such as the rat ovarian weight method, can be adapted, by examination of the histology of the stimulated ovaries, to differentiate to some extent between different types of stimulation. Hypophysectomized animals may be used rather more successfully for the same purpose, but no great precision can be expected with a mixed preparation.

We are thus faced with no adequate method for the separate estimation of FSH or of LH, and with the necessarily rather unsatisfactory combined measurement of the two together. Interest does not currently centre on PMS or chorionic gonadotrophin, so it is fortunate that chromatographic separation (Crooke, Butt, Ingram & Romanchuck, 1954) now promises better things with pituitary hormones, even in small amounts.

Early work by Fevold (1937, 1939) and his co-workers (Fevold, Hisaw & Greep, 1937) showed that, in the rat ovary, FSH alone causes a limited weight increase due to follicular growth, LH alone causes none, while FSH and LH in varying combinations act synergistically and give higher ovarian weights than FSH alone. Similar tests are possible with the seminal vesicles in males. Much other work along similar lines has been carried out, mostly with the conclusion that hypophysectomized animals must be used, although Steelman & Pohley (1953) have recently advocated the use of intact female rats in a detailed study of the assay of FSH based on augmentation of the response to chorionic gonadotrophin (see fig. 5).

Evans, Simpson, Tolksdorf & Jensen (1939) used immature female hypophysectomized rats and showed that FSH alone causes the growth of many follicles without luteinization, whereas LH alone causes the disappearance of the characteristic deficiency cells (" wheel cells ") in the thecal tissue. In the male hypophysectomized when immature, FSH stimulates only the germinal tissue with no androgen production, while LH causes interstitial cell growth, androgen production, and

FIG. 5. RESPONSE OF THE IMMATURE RAT OVARY TO FOLLICLE-STIMULATING HORMONE (FSH) AUGMENTED BY 20 i.u. OF CHORIONIC GONADOTROPHIN (Steelman & Pohley, 1953)



Abscissae: dose of FSH (mg.)
Ordinates: ovarian weight (mg.)

Groups of 6 rats were used.

consequent stimulation of secondary sexual organs. Nalbandov, Meyer & McShan (1946) have shown that hypophysectomized cocks are particularly useful for differentiating the FSH and LH content of a preparation, since FSH alone causes testicular enlargement but no comb growth, whereas LH with or without FSH causes both. Chorionic gonadotrophin was without effect.

If immature female rats or mice are used, some indication of the FSH and LH content of extracts may be gained from the combined evidence of ovarian and uterine weight increases, together with the nature of ovarian changes. Care must be taken that such assays are not invalidated by the presence of oestrogens, which may occur in extracts prepared by precipitation techniques (cf. Palmer, 1937). Hamburger (1950) recommends the use of 21-day old female mice for the examination of urine extracts. They are injected five times in two days and killed four days from the start of the test. The ovaries are weighed and examined histologically, or by macroscopic inspection. Zondek & Sulman (1945, 1948) describe a rapid test, mainly for LH, in which ovarian hyperaemia is induced in the rat. The test takes 24 hours and has the drawback that not all strains of rat are responsive. The various toad and frog tests which utilize the production of ovulation or liberation of spermatozoa have been almost exclusively investigated as tests of pregnancy and for the estimation of chorionic gonadotrophin.

Some of the above methods may be used as valid quantitative assays in certain circumstances—as in the assay of postmenopausal urine, which contains an excess of FSH, or the assay of pregnancy urine, which usually contains a vast excess of chorionic gonadotrophin. They may then give accuracies of the order of 80–125% ($P = 0.95$) with 50–100 mice per assay, fewer if litter-mates are used in an efficient design. The vaginal-smear test, dependent on the induced oestrogen production that occurs when all but pure FSH is used, has been found particularly accurate in some hands (Emmens, 1939b, 1939c).

Luteotrophic activity has never been assayed as such, but there are some semi-quantitative tests. For discussion of some of these Astwood (1953) may be consulted. The identity of luteotrophin, prolactin, and lactogenic hormone is, as Astwood points out, not yet fully proven but seems very likely. Until we are certain, however, a quantitative test for luteotrophic activity as such is needed. An assay method could presumably be developed from the known effects of luteotrophic stimulation—prolongation of corpus luteum function, inhibition of responses to oestrogens, deciduoma formation, and other consequences of progesterone release, but none is available.

The assay of prolactin as a crop-gland stimulating agent, whether it be considered a gonadotrophin or not, is beyond the scope of this article. It should be noted, however, that the assay of urinary prolactin activity is feasible (cf. Segaloff, 1953) by the crop-gland stimulating technique.

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CLINICAL VALUE OF HORMONE ESTIMATIONS

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1 Gonadotrophins
2 Progesterone and its metabolites
3 Oestrogens
4 Summary
References

Assays of the reproductive hormones, which include gonadotrophins, oestrogens, and progesterone and its metabolites, have been used for many years as diagnostic procedures in the field of obstetrics and gynaecology. Much information has accumulated, and it is pertinent at this time to re-evaluate the clinical use of these assays.

It is now well recognized that many factors influence bioassays, such as the strain of animal, number of injections and route of administration, and comparable results are not always obtained by workers in different laboratories (Emmens, 1939). For this reason it is important that each laboratory should establish standards of normality for the methods employed, and that results should be expressed in terms of an international standard. Similarly, when chemical methods are used for the assay of hormones, the normal range of values should be established for the method used.

The interpretation of any hormone assay requires a fundamental knowledge of the physiology upon which the tests are based and of the variations that occur from day to day in hormone secretions. A sufficiently detailed history of the patient should be available, and results should be considered only in close association with the clinical findings.

Gonadotrophins, oestrogens and progesterone are elaborated into the blood stream and after metabolism in the body are excreted in the urine. Because of the limitations in using blood, the majority of assays have been applied to urine. This necessitates the collection of urine over a period of 24 hours, as the hormones are not excreted at a constant rate throughout the day and night. The completeness of this collection should always be checked by creatinine determinations.

Investigations carried out on administered hormones show that these substances disappear rapidly from the blood stream, and only small amounts are recovered in the urine. The liver is one of the most active organs concerned with the degradation and conjugation of the steroid hormones, but whether endogenously produced hormones are inactivated and destroyed as rapidly as administered hormones is still unknown.

Little is known regarding the manner in which the hormones exert their influence on body tissues. While the excretion of hormones in the urine may reflect to some extent the secretion of these substances by the various organs involved, the amount excreted does not necessarily indicate the amount that has been utilized.

1. Gonadotrophins

In man, three different kinds of gonadotrophin may be excreted in the urine, and various methods of assay have been developed in order to differentiate their activities.

The two active principles formed in the anterior pituitary gland are the follicle-stimulating hormone (FSH) and the luteinizing (LH) or interstitial cell stimulating hormone (ICSH). The third, chorionic gonadotrophin, is produced by the actively growing trophoblast of the chorionic epithelium of the placenta. Chorionic gonadotrophin is present in the blood and urine of pregnant women. Human chorionic

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gonadotrophin may also be produced by any rapidly growing chorionic tissue, regardless of its site in the body. In man chorionic gonadotrophin has a luteotrophic action.

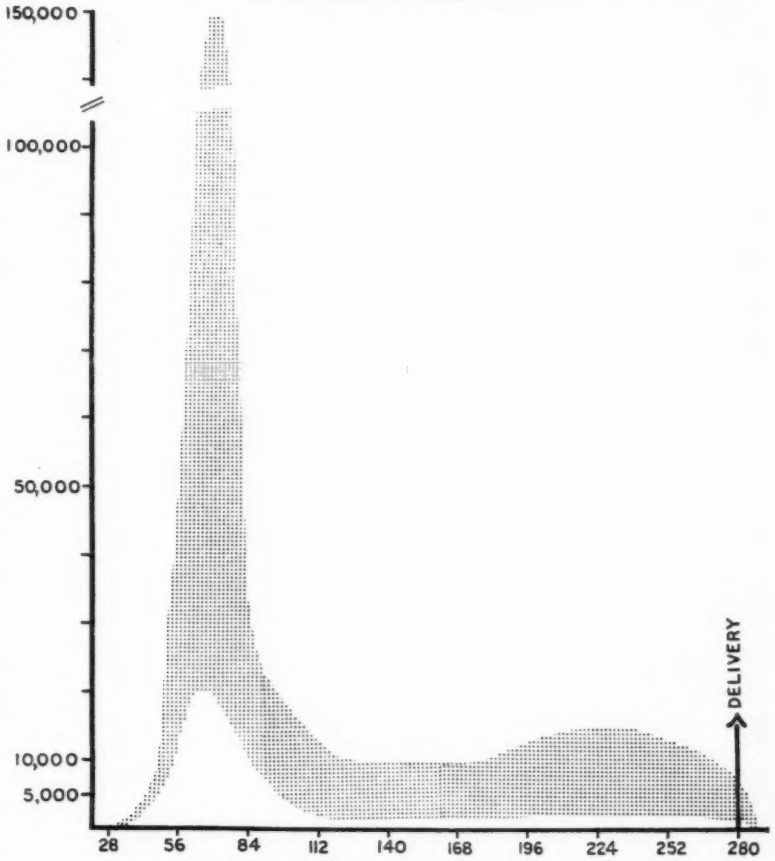
FSH and LH. The assay of FSH has been widely used in clinical medicine for the characterization of primary and secondary ovarian failure (Sturgis, 1950). Although this assay is of great diagnostic value it must be realized that accurate measurements of FSH are not possible by the methods generally employed. It is difficult to obtain extracts of pure FSH from urine and, as FSH and LH act synergistically, the presence of small amounts of LH will greatly enhance the action of FSH on the ovaries of the test animal. In order to distinguish between the two pituitary fractions, it is necessary to compare their relative effects on both ovarian weight and on ventral prostatic weight in hypophysectomized animals. In male hypophysectomized rats, LH or ICSH causes an increase in the weight of the ventral prostate and seminal vesicles. According to McArthur (1952) there is no synergistic effect of FSH and ICSH on the weight of these organs. In clinical laboratories the method most commonly used for the assay of FSH is dependent upon the increase in uterine and ovarian weight in immature mice. This assay will be affected by the presence of small amounts of LH in the extracts. In normal non-pregnant women there is a continuous excretion of gonadotrophin throughout the menstrual cycle, with increased amounts occurring at the time of ovulation and occasionally in the latter part of the luteal phase. This gonadotrophin is a mixture of varying amounts of FSH and LH. According to Klinefelter, Albright & Griswold (1943), normal menstruating women excrete from 6.6 to 53 m.u./24 hr. When ovarian function fails and oestrogen secretion decreases to low levels, the pituitary elaborates gonadotrophins in increasing amounts, and in post-menopausal urine greater amounts of FSH are found.

In spite of the criticism which may be levelled at the method used for the clinical assay of gonadotrophin, this assay may be most helpful in the classification of the various causes of amenorrhoea. When amenorrhoea is due to primary ovarian failure an increase in gonadotrophin is associated with a low oestrogen output. When ovarian failure is secondary to pituitary failure, low gonadotrophins and low urinary oestrogens will be found. In some patients amenorrhoea may occur in the presence of normal amounts of gonadotrophins. It has been suggested (Klinefelter *et al.* 1943) that in these cases there may be an interference in the hypothalamic release of LH, with a consequent decrease in oestrogen production.

The assay of gonadotrophin is also helpful in evaluating pituitary function in those cases of panhypopituitarism in which all the trophic factors are decreased.

Chorionic Gonadotrophin. Chorionic gonadotrophin appears in the urine almost immediately after implantation of a fertilized ovum. The rate of excretion rises rapidly to reach a maximum about 50–70 days after the beginning of the last actually occurring menstrual period. Our range of values found throughout 10 normal pregnancies is shown in fig. 1. According to other investigators the amount of chorionic gonadotrophin excreted at the peak may range from 20,000 to 450,000 i.u./24 hr. Recently Loraine (1950), using a method of assay depending upon increase in prostatic weight in male rats, found in four cases of normal pregnancy 20,000–40,000 i.u. of chorionic gonadotrophin/24 hr. between the 40th and 80th day of pregnancy. Usually by the 120th day the levels of chorionic gonadotrophin have decreased to values ranging from 3,000 to 10,000 i.u./24 hr. Thereafter the level of excretion in most cases remains relatively constant until parturition, after which it declines to non-pregnant levels in 5–10 days. The concentration of chorionic gonadotrophin in the serum

FIG. 1. RANGE OF EXCRETION OF CHORIONIC GONADOTROPHIN IN NORMAL PREGNANCY



Abscissae: days after beginning of last menstrual period
Ordinates: excretion of chorionic gonadotrophin (i.u./24 hr.)

The assay was based upon the production of a full squamous response in the vaginal smear of 21-day-old rats.

follows the curve observed in the urine. At the peak of excretion the serum contains 200–600 i.u./ml. of chorionic gonadotrophin (Smith, Albert & Randall, 1951).

In order to shorten the time required for the assay of gonadotrophin and to simplify the procedure, many new tests have been developed for the assay of chorionic gonadotrophin since Aschheim & Zondek (1928) first described their pregnancy test. Reports have been published which show that the reliability of these new pregnancy tests compares favourably with that of the Aschheim-Zondek test (Crew, 1939; Landgrebe & Hobson, 1949). In 600 cases we found an agreement of 97% between the Aschheim-Zondek and the six-hour hyperaemia pregnancy tests. When these methods were used quantitatively the agreement was less consistent.

Diagnosis of early pregnancy. The assay of chorionic gonadotrophin in urine or in serum has proved to be of great value in detecting early pregnancy. Pregnancy tests are so designed as to give a positive reaction at levels of gonadotrophin much higher than those found in the non-pregnant state. In early pregnancy these tests become positive between the 35th and the 40th day after the beginning of the last menstrual period. The increase in excretion of chorionic gonadotrophin that takes place at the beginning of pregnancy does not occur at the same time in all women, nor at the same rate in relation to the last period. A false negative reaction may be obtained when the specimen is collected too early in pregnancy, and where ovulation has been delayed and the concentration of hormone has not reached the threshold value of the test. If this is suspected, then the test should be repeated after an interval of one week. A recent survey has shown that the concentration of gonadotrophin may vary considerably in the first morning specimen of urine. It is therefore advisable to measure the specific gravity of the urine before proceeding with the assay. The insensitivity of the test animals may also be responsible for false negative tests. Pregnancy tests when applied to urine obtained in late pregnancy may not be reliable, because of the low amounts of gonadotrophin occasionally excreted at this time.

In certain conditions other than pregnancy there is an increase in the secretion of pituitary gonadotrophins which may occasionally give rise to false positive tests. These include the menopause, corpus luteum or follicular cysts, uterine cancer and squamous-cell carcinoma of the cervix.

Threatened abortion. One of the many causes responsible for the condition of threatened abortion is the failure of the trophoblast to secrete increasing amounts of chorionic gonadotrophin, the function of which is to stimulate the ageing corpus luteum until the trophoblast has increased its own progesterone production to the maintenance level. A low output of chorionic gonadotrophin is associated with a dying trophoblast and is usually followed by abortion. A normal output of chorionic gonadotrophin will not, however, always preclude impending abortion, as the corpus luteum of pregnancy may not respond to the increased stimulus or the placenta may be too slow in the elaboration of progesterone and oestrogen; these conditions would also lead to abortion. In threatened abortion in late pregnancy, the assay of progesterone metabolites and oestrogens may be more helpful in the prognosis than that of chorionic gonadotrophin.

Pregnancy tests are not reliable in ectopic pregnancy. A positive pregnancy test will be obtained only as long as functioning chorionic epithelium is in contact with the blood stream. If the chorion becomes separated from the maternal circulation by fibrous tissue or by haemorrhage, pregnancy tests will become negative.

Death of foetus. The assay of chorionic gonadotrophin is not a satisfactory guide in attempts to determine the viability of a foetus. As the elaboration of chorionic gonadotrophin is a function of the trophoblast, a positive pregnancy test does not necessarily imply that the foetus is alive. For instance, in cases of missed abortion, the excretion of chorionic gonadotrophin will continue until degeneration of the placenta occurs. If the pregnancy test becomes negative, this evidence would point to the death of the foetus, although it must be borne in mind that low excretions of gonadotrophin may occur in the latter part of pregnancy.

Hydatidiform mole and chorionepithelioma. Both these tumours elaborate large amounts of chorionic gonadotrophin, and it is generally believed that these conditions can be differentiated from a normal pregnancy by means of a quantitative assay of this hormone. A differential diagnosis can be made only when the level of gonadotrophin in the blood, or the amounts excreted in the urine, exceed those found between the 50th and 80th days in normal pregnancy. The curve of excretion of chorionic gonadotrophin in cases of hydatidiform mole and chorionepithelioma differs from that of a normal pregnancy in that the levels of the hormone continue to rise. Wide variations occur in the amounts of hormone elaborated by different tumours, which depend to some extent upon their age. In some cases small amounts of pregnanediol and oestrogens are found in the urine (Hinglais & Hinglais, 1949). Following the expulsion of a mole, serial quantitative studies on the excretion of chorionic gonadotrophin should be made every two to four weeks until levels have decreased to normal values. A rising titre of gonadotrophin at this time usually indicates the presence of a malignant disease.

Toxaemia of pregnancy. Smith & Smith (1937) reported the finding of increased amounts of gonadotrophin in the blood of patients with toxaemia of pregnancy. The increase in serum gonadotrophin preceded clinical signs by four to eight weeks. As a result of these studies White & Hunt (1943) investigated a large series of cases of pregnancy complicated by diabetes and reported that a rising serum gonadotrophin gave a poor prognosis and an indication for hormonal therapy.

Loraine (1949) in a recent study found no definite correlation between the chorionic gonadotrophin excretion and the insulin requirements in a group of 14 pregnant diabetics—only six of these patients showed a higher than normal excretion of gonadotrophin. Oestrogen therapy caused only a temporary decrease in the output of gonadotrophin.

2. Progesterone and its Metabolites

Because of the low concentration of progesterone in body fluids, the assay of this hormone has had little clinical application. Instead, the measurement of the metabolic products of progesterone, pregnanediol and pregnanolone, has been widely used as an index of the production of progesterone by the corpus luteum and the placenta.

Pregnanediol is also the metabolic end-product of certain adrenal steroids, and small amounts are present normally in the urine of men and women. These amounts are usually too low to be determined by the various methods used for the routine estimation of pregnanediol. In certain cases of hyperactivity of the adrenal, pregnanediol can be demonstrated in the urine, and caution must be used in interpreting this finding as a reflection of luteal activity.

Pregnanediol and pregnanolone are excreted in the urine as glucuronides, and various methods have been developed to measure these steroids either as conjugated material or free steroid (Venning, 1937, 1938; Sommerville, Gough & Marrian, 1948). Both types of methods are satisfactory as clinical procedures.

Ovulation and length of luteal phase. The presence of pregnanediol in the latter part of the cycle of menstruating women has been taken as indirect evidence for the occurrence of ovulation. At the present time, a simpler procedure, based upon the mid-cycle rise in basal body temperature, is in use in many clinics. Attempts to correlate basal body temperature, pregnanediol excretion and appearance of the endometrium (Rogers & Sturgis, 1950; Buxton & Engle, 1950) indicate that the time of ovulation determined by these various indices may differ by several days.

A shortened luteal phase has been suggested as one of the many causes of sterility in women. Daily assays of pregnanediol, from the tenth day of the cycle until bleeding appears, will determine the length of the luteal phase and reflect the amount of progesterone elaborated by the corpus luteum. Although the critical amount of progesterone necessary for the production of an endometrium that will allow implantation of a fertilized ovum has not been established with certainty, total excretion values of pregnanediol of below 20 mg. throughout the luteal phase are generally considered inadequate.

Pregnancy test. The use of pregnanediol assays as a means of establishing early pregnancy was first advocated by Hain & Robertson (1939). Since that time Guterman (1944) has developed a pregnancy test based upon the excretion of pregnanediol in urine. This test has been evaluated by Bender (1948), Cope (1940), Henderson, MacLagan, Wheatley & Wilkinson (1949), Merivale (1948), Swyer (1949) and many others. It is generally agreed that the assay of pregnanediol for the detection of early pregnancy is not as reliable as that of chorionic gonadotrophin, as there is an overlapping of values for pregnanediol excretion in the luteal phase and in early pregnancy. The test may be of use where animal facilities are not available. At least two determinations should be made, with a sufficient interval of time between them to exclude a late luteal phase.

Threatened abortion. In fig. 2 is shown the curve of excretion of pregnanediol as determined from a large series of normal pregnant women (Venning, 1948). In early pregnancy the pregnanediol excretion is maintained within narrow limits at or slightly above the maximum attained during the luteal phase for a period of approximately eight weeks. The excretion then increases at a more rapid rate and reaches a maximum in the last month of pregnancy. At this time, pregnanediol levels range from 40 mg. to 130 mg./24 hr. in different women during normal pregnancy.

The cause of abortion is by no means due to a single entity. In some cases it may be the result of corpus luteum failure or delayed placental production of reproductive hormones. In patients with symptoms of threatened abortion, the finding of a repeated low or absent pregnanediol in the urine is evidence of abnormality in the luteal or placental function. Although it is possible for a pregnancy to continue (despite low levels of pregnanediol in the early stages), this situation is uncommon. If the pregnanediol is absent on two or more occasions, then abortion usually follows.

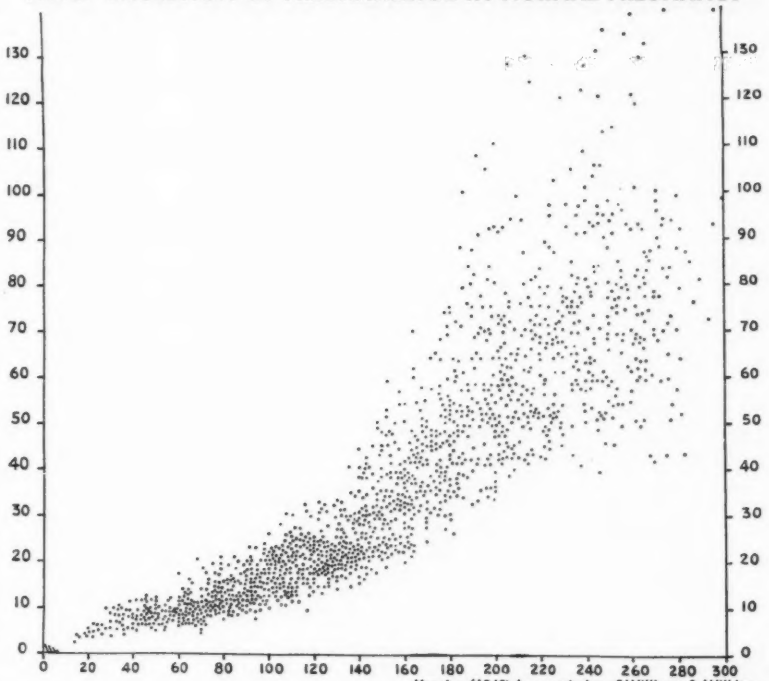
Even if pregnanediol is present in normal amounts in early pregnancy, the possibility of abortion is not always precluded. If the patient has a history of recurrent abortions, it is useful to follow the pregnanediol excretion at short intervals over the critical period. A progressive decline in the output of pregnanediol implies a poor prognosis.

Diagnosis of death of the foetus. Pregnanediol output is a function of the corpus luteum and the placenta, and a normal amount of pregnanediol in the urine is not evidence of the viability of the foetus. If death of the foetus is suspected, repeated determinations at intervals of a week are of value. A negative pregnanediol excretion or a gradual decrease in the output of this steroid would be evidence in support of this diagnosis.

3. Oestrogens

Three oestrogens have been isolated from human tissue and body fluids. They are oestradiol, oestrone and oestriol.

FIG. 2. EXCRETION OF PREGNANEDIOL IN NORMAL PREGNANCY



Abscissae: days of pregnancy
Ordinates: excretion of pregnanediol (mg./24 hr.)

These hormones differ greatly in their biological activity, oestradiol being the most potent. Oestradiol and oestrone are interconvertible in the body and both hormones are degraded to oestriol. All three are found in urine in the form of conjugates. Oestrogens are secreted by the ovary and the placenta, and there is evidence that the adrenals and testes also elaborate oestrogenic substances. Because of the small amounts of oestrogens excreted by the non-pregnant woman, biological assays are generally used for their determination (Emmens, 1948). The Allen-Doisy test, based upon changes that occur in the vaginal smear of rats or mice, has had the widest application. It must be recognized that when biological assays are applied to crude urinary extracts in which there is a mixture of oestrogens, only total activity can be measured; no information regarding the total weight of oestrogen is obtained by this assay. Fractionation of the oestrogenic material into oestradiol, oestrone and oestriol fractions is necessary for determining total amounts. This separation is not usually carried out as a routine laboratory procedure. Colorimetric and fluorimetric methods for oestrogens have been developed but these are applicable only to urines containing large amounts of oestrogen. In pregnancy there is a gradual increase in the output of oestrogen, and in the last month 20–50 mg./day are excreted.

Because of the wide fluctuation in the excretion of oestrogens and the difficulties involved in their measurement, assays have had a limited application in clinical medicine. In cases of amenorrhoea, assays of oestrogens and gonadotrophins are of value in the differential diagnosis of pituitary or ovarian failure.

Oestrogen excretion is increased in the presence of functioning ovarian tumours such as granulosa-cell tumour and thecoma. It is now believed that oestrogens are produced only by thecal cells and that it is these cells in the granulosa-cell tumour that are responsible for the increased oestrogen secretion. Oestrogen titres, determined colorimetrically, have been suggested as tests for pregnancy. There is an increase in the concentration of urinary oestrogen in early pregnancy; the level is not high enough, however, to allow an accurate chemical assay to be made at this stage of pregnancy.

4. Summary

The assay of the reproductive hormones is of diagnostic value in establishing the presence of an early pregnancy and of hormonal imbalance in a variety of conditions. These assays should be considered only in conjunction with the clinical findings, and in relation to the limitations of the methods employed.

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HORMONES IN MAMMARY GROWTH AND FUNCTION

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1 Growth of the mammary gland
  a Normal growth
  b Experimentally induced growth
2 Initiation of lactation (lactogenesis)
  a Biochemistry of lactogenesis
  b Initiation of lactation at parturition: hormonal regulation
3 Maintenance of lactation: galactopoiesis
  a Somatotrophin
  b Prolactin
  c Adrenocorticotrophin
  d Pituitary-thyroid axis
  e Endocrine pancreas
References

The present topic was reviewed in its various aspects in a previous issue of this Bulletin dealing with lactation (*Brit. med. Bull.* 1947, Vol. 5, No. 2-3), to which the reader is referred. These reviews must serve as the starting-point for the present brief sketch of some recent advances, which is all that is possible in the space available. In selecting the topics to be discussed, preference has been given to work carried out within the British Commonwealth; in general it has been possible to mention other work only where necessary to establish the background. Since the above-mentioned issue of this Bulletin appeared, detailed reviews of the hormonal control of the mammary gland have been published by Folley & Malpress (1948a, 1948b), Mayer & Klein (1948, 1949) and Folley (1952a, 1954). The more biochemical aspects of the hormonal control of the mammary gland were reviewed by Folley (1951, 1952c).

1. Growth of the Mammary Gland

a. Normal Growth

Recently there has been a quickening of interest in the development of quantitative and objective methods of studying mammary growth, probably because it is recognized that the subject has reached a stage of development such that the qualitative methods that have served so well in the past are no longer adequate to ensure further progress. Many of these methods have been reviewed by the present author (Folley, 1952b); others have subsequently been developed in our laboratory by Silver (1953a) and Flux (1954a).

Quantitative studies, in our laboratory, of the rate of increase in total mammary gland area in relation to the increase in body-size in the rat and mouse have provided new information about the time relations of mammary development. Cowie (1949) found that in the female Norway rat the total mammary area increased isometrically with body surface from birth until about the 21st day, when, provided the ovaries were intact, a phase of positive allometric growth ($\alpha = 3.0$)[1] set in. In animals ovariectomized at the 21st day, isometric growth (α approximately unity) continued. These findings were later confirmed in their essentials in our laboratory by Silver (1953a), who further found that ovariectomy 12 days earlier was followed by a phase of allometric growth ($\alpha = 1.45 \pm 0.09$). This might be due to the action of mammogenic steroids secreted by an adrenal cortex under unremitting stimulation by a pituitary released from ovarian restraint. Flux (1954a) has since studied the change-over from isometric to allometric mammary area increase in mice of Strong's CHI strain, with essentially similar results, save that for the allometric phase, which began at about the 24th day, α had the value 5.2; this shows that in the CHI mouse the mammary-duct system grows much faster in relation to body surface than it does in the Norway rat. In the latter the onset of the allometric phase precedes vaginal opening and the initiation of oestrous cycles (i.e. puberty as usually understood) by a good many days, but this is not so in the CHI mouse, in which oestrous cycles begin at about the 28th day, only a few days after the allometric phase of mammary growth begins.

The necessity of the ovary for the induction of the allometric phase is clear from these results, but the mechanism involved and the reason why allometric mammary growth begins just when it does are intriguing but still largely unsolved questions. The studies of Silver (1953b) and Flux (1954a) have, however, illuminated them to some extent. Silver (1953b) found that the mammae of suckling rats were unresponsive to oestrogen administered between the 11th and 19th days of age unless an unfractionated extract of ox anterior pituitary was administered at the same time; evidently the pituitary synergism, which all agree is necessary for complete mammary growth, was lacking in these young rats. But we still do not know if maturation of the pituitary gland of the young animal had not gone far enough or if the secretion or action of the synergistic hormones was prevented by an inhibiting substance present in the mother's milk. A milk-borne inhibitor seems unlikely, since Flux (1954a) found that weaning at 15 days did not affect the relative rate of growth of the mammae of mice. But we must note that he also found the mouse different from the rat in that while its mammae were unresponsive to oestrogen from the 10th to 15th day of life (though oestrogen and pituitary extract together were effective) they responded to oestrogen after the 15th day.

b. Experimentally Induced Growth

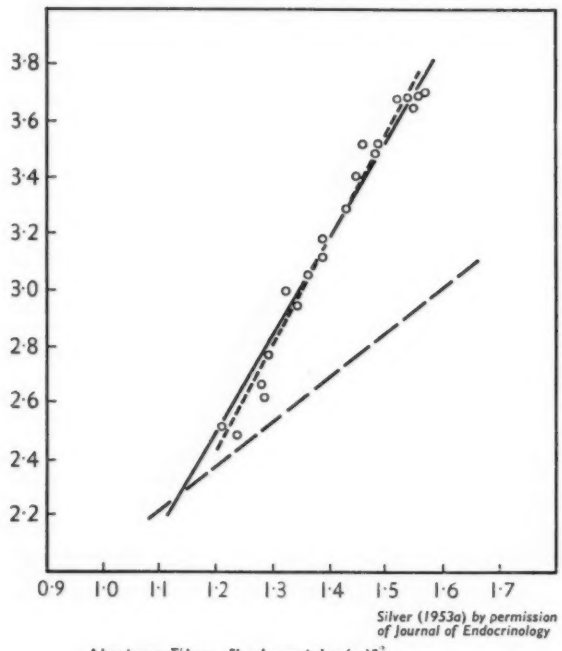
The animals commonly used for experiment can be divided into three broad categories, according to whether oestrogen in the absence of progesterone induces relatively little alveolar growth, as in the mouse, rat and rabbit, whether without progesterone it causes extensive lobule-alveolar growth, as in the guinea-pig, cow and goat, or finally, whether in the absence of progesterone it induces only slight growth of the mammary ducts, as in the dog (Trentin, De Vita & Gardner, 1952).

As regards the first of these categories, some indication of the levels of oestrogen which may circulate in the body during the allometric phase of mammary growth has been

[1] α denotes the constant of allometry or equilibrium constant. For terminology of relative growth and other information the reader should consult Huxley & Teissier (1936).

obtained by Silver (1953a) for the rat and by Flux (1954a) for the mouse. Silver (1953a) was best able to mimic ($\alpha = 3.70 \pm 0.13$) the normal relative rate of mammary duct growth ($\alpha = 3.40 \pm 0.17$) by injecting 0.1 μg. of oestradiol dipropionate every other day from the outset (40 g. rat) and then increasing the dose step-wise with the body-weight (see fig. 1). She also showed that folic acid is probably concerned with the growth response of the mammary ducts to oestrogen since, as shown in fig. 2, the folic acid antagonist, aminopterin, decreased the relative rate of increase in mammary area in response to injected oestrogen (Silver, 1954). Flux (1954a) found that, in the mouse, 0.01 μg. of oestrone given daily doubled the total mammary area in spayed mice over that of spayed controls; this suggests that the threshold dose must be somewhat lower. Higher doses (0.055 μg./day) were necessary to attain the total mammary areas characteristic of comparable intact mice. In a study of the role of the adrenal cortex in mammary growth in the mouse, Flux (1954b) found that glucocorticoids inhibited the duct growth response to oestrogen, whereas deoxycorticosterone, on the other hand, had an essentially opposite effect.

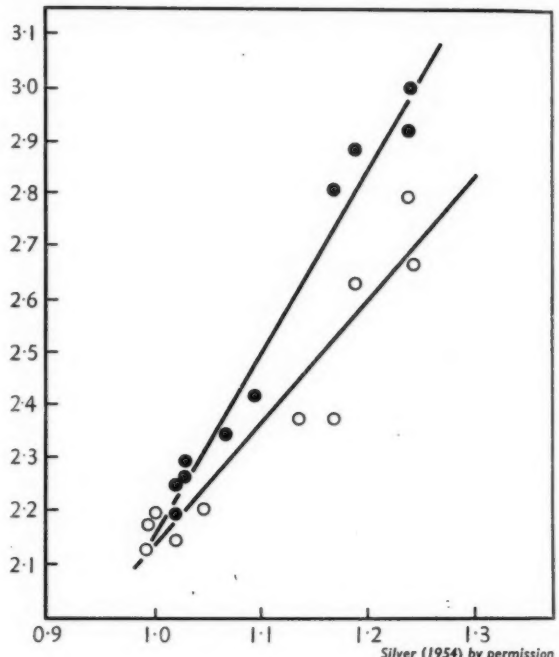
FIG. 1. RELATION BETWEEN TOTAL AREA OF MAMMARY GLANDS AND "BODY SURFACE" (BODY-WEIGHT$^{\frac{2}{3}}$) IN THE OVARIECTOMIZED RAT (INITIAL WEIGHT 40 g.) RECEIVING OESTRADIOL DIPROPIONATE, BEGINNING WITH 0.1 μg. ON ALTERNATE DAYS, AND INCREASING BY 0.1 μg. FOR EVERY 10 g. INCREASE IN BODY-WEIGHT



Silver (1953a) by permission of Journal of Endocrinology

Abscissae: $\frac{2}{3}\log_{10}$ [body-weight (g.)]$^{\frac{2}{3}}$
Ordinates: $\frac{2}{3}\log_{10}$ area of mammary glands (mm.^{2})
o------o rats receiving oestrogen ($\alpha = 3.70 \pm 0.13$)
——— regression curve for intact controls ($\alpha = 3.40 \pm 0.17$)
— — — regression curve for ovariectomized controls ($\alpha = 1.45 \pm 0.09$)

FIG. 2. RELATION BETWEEN TOTAL AREA OF MAMMARY GLANDS AND "BODY SURFACE" (BODY-WEIGHT$^{\frac{2}{3}}$) IN CONTROL AND AMINOPTERIN-TREATED OVARIECTOMIZED RATS RECEIVING 0.25 μg. OF OESTRADIOL DIPROPIONATE ON ALTERNATE DAYS



Silver (1954) by permission of Journal of Endocrinology

Abscissae: $\log_{10}$ [body-weight (g.)]$^{\frac{2}{3}}$
Ordinates: $\log_{10}$ area of mammary glands (mm.^{2})
o——o rats receiving aminopterin + oestradiol dipropionate ($\alpha = 2.29 \pm 0.08$)
●——● pair-fed controls receiving oestradiol dipropionate ($\alpha = 3.45 \pm 0.06$)

Turning now to species of the second category, we find that recent studies of experimental mammary development and induced lactation in farm animals (see Malpress, 1947, and Cowie, Folley, Malpress & Richardson, 1952, for reviews of the earlier work) have substantiated the contention of Mixner & Turner (1943) that, though oestrogen by itself causes extensive alveolar development in these animals, the alveolar histology is far from normal unless the oestrogen is reinforced (or ?counterbalanced) by sufficient progesterone. Using special techniques (developed by K. C. Richardson) for quantitative study of the structure of the goat udder, Cowie *et al.* (1952) have shown that udders grown by hexoestrol in spayed virgin goats exhibit a variety of histological abnormalities. Of these, probably the one most significant functionally was a deficiency in the total alveolar surface area (see also Richardson, 1953); this was most likely due to the alveoli being too large compared with those of the normal goat udder. Richardson (1953), in further examination of this material, has since confirmed that conveyance of progesterone along with the oestrogen significantly increased the alveolar surface area per unit volume; this means that the

hormone combination gave smaller alveoli, which probably fall within the normal size range. Despite the exceptionally good yields reported by Reineke, Meites, Cairy & Huffman (1952) in sterile cows brought into lactation by oestrogen plus progesterone, there is no good evidence yet that the combined treatment gives significantly better yields than oestrogen alone.

The nature of the role of the anterior pituitary in mammary growth, a question the controversial nature of which is apparent on reference to a previous review in this Bulletin (Folley, 1947a), has been somewhat clarified by recent work. This indicates that in the rat at least, while, as all will agree, pituitary intervention is necessary for normal mammary alveolar growth, the hypophyseal influence is mediated through one or more of the six well-characterized pituitary hormones, rather than through specific pituitary mammogens as required by the " mammogen " theory of C. W. Turner. This conclusion follows from recent studies on mammary growth in the hypophysectomized female rat by Lyons (1951), Lyons, Li & Johnson (1952) and Lyons, Li, Cole & Johnson (1953), which show that, in the pituitary-less and spayed rat, mammary growth equivalent to that of the second half of pregnancy can be evoked by treatment with oestrogen and progesterone together with the purified anterior pituitary hormones, prolactin and somatotrophin. It would thus appear that, as regards the rat at least, there is no need to postulate the existence of specific pituitary mammogenic hormones. This probably also applies to other species.

2. Initiation of Lactation (Lactogenesis)

a. Biochemistry of Lactogenesis

The ingenious discovery of Lyons (1942), who showed that minute quantities of prolactin injected directly into the teat galactophores of the non-lactating rabbit with prepared mammae caused localized lactational responses, has been confirmed by Meites & Turner (1948) and more recently in our laboratory by T. R. Bradley, who has made some progress towards using this response as the basis of an assay method for prolactin that may possess important advantages over the pigeon-crop assay.

These experiments suggest that the lactogenic action of prolactin is exerted directly on the mammary epithelial cells, but none of them excludes the possibility that other anterior pituitary hormones (for example, adrenocorticotrophin) are involved in a pituitary " lactogenic complex ", as suggested by Folley & Young (1941), since the experimental animals were left with intact pituitaries capable of synergistic action. (The concept of " permissive " action (see Ingle, 1954) in this context I leave aside as being to me philosophically meaningless.) In this connexion it is worth noting that Lyons *et al.* (1953) report that lactation could be initiated in their hypophysectomized rats possessing experimentally developed mammae by injection of the " miraculous triad ", prolactin, somatotrophin and cortisone (or adrenocorticotrophic hormone (ACTH)), together with thyroxine. The intraduct injection experiments suggest that it should be possible to evoke in vitro, in isolated mammary tissue taken from rats killed in late pregnancy, such biochemical changes as are characteristic of lactogenesis. The work of Folley & French (1949b) shows that lactogenesis in the rat is accompanied by an increase in the power of the tissue to utilize glucose for various purposes, among them energy production and lipogenesis, as shown by the facts that mammary tissue from post-parturient, but not pregnant, rats exhibits a Q_{O_2} in glucose markedly above endogenous values, and that the R.Q. (in glucose), which for tissue from pregnant animals is < 1, after parturition rises to values > 1. As a consequence of their high R.Q., slices of mammary gland from lactating rats incubated in Warburg vessels in bicarbonate-saline with suitable substrates—the atmosphere being a mixture of O_2 and CO_2—give rise to a steady increase in net pressure, i.e., the composite respiration curve (CRC) has a positive slope. The gas exchange of mammary slices from pregnant rats, on the other hand, is usually such that the CRC has, if anything, a slight negative slope (Balmain & Folley, 1952). Balmain & Folley (1952) reported preliminary experiments indicating that addition of purified prolactin to the incubation medium had no effect on the slope of the CRC of mammary slices from pregnant rats, though it did increase the slope of the CRC of slices taken from rats in early lactation. Whether or not this latter can be interpreted as a lactogenic, rather than galactopoietic, effect it is difficult to say. It has been confirmed by T. R. Bradley in more extensive studies (largely unpublished) in our laboratory, but since the concentration of prolactin required proved to be rather high, and similar effects were given by some (but not all) preparations of somatotrophin and adrenocorticotrophin examined, it was felt that a contaminant might be responsible. A study carried out in collaboration with F. W. Landgrebe and G. M. Mitchell (Bradley, Folley, Landgrebe & Mitchell, 1954) indicated that intermedin was particularly active (in concentrations down to 1.25 μg./ml.) in increasing the slope of the CRC of mammary slices from the lactating rat, and we then concluded that the effect observed with prolactin might well be due to contamination with intermedin. However, more recently Bradley (unpublished work) has found a prolactin preparation which will increase the slope of the CRC of mammary slices from rats in late pregnancy, upon which intermedin is ineffective. It would thus appear that an effect of prolactin in vitro has now been observed, which on the face of it should merit the description " lactogenic ". These matters obviously need further study.

b. Initiation of Lactation at Parturition: Hormonal Regulation

Some recent work is pertinent to the theories as to how copious lactation is initiated at parturition that were discussed previously in the Lactation number of the Bulletin (Folley, 1947b). This discussion was mainly concerned with critical examination of the ingenious theory advanced by Meites and Turner in 1942, for which further advocacy has been made by Meites & Turner (1948). However, Meites & Sgouris (1953, 1954) have recently evoked mammary growth in gonadectomized rabbits by administration of oestrogen and progesterone, and found that continuation of this regime prevented the initiation of lactation by prolactin given in doses which were effective in the presence of either steroid by itself. This finding is clearly not in harmony with the Meites-Turner theory, according to which prolactin should have been just as effective lactogenically in the presence of both steroids as in the presence of either alone. Results obtained in our laboratory (Cowie *et al.* 1952) in experiments on hormonally induced alveolar growth and lactation in the goat also point to a combination of oestrogen and progesterone as being a particularly potent inhibitor of lactation (probably the hormonal influence which holds lactation in check during pregnancy), a concept which dates back

originally to the work of Fauvet (1941). Fauvet appears to have been the first to show that oestrogen and progesterone, in doses ineffective by themselves, in combination effectively inhibited lactation in spayed female rats. These and other considerations have prompted the present author (Folley, 1954) to advance a tentative theory of the mechanism of induction of lactation that combines features of earlier hypotheses and seems capable of harmonizing most of the known facts. This working hypothesis can be summarized under the following heads.

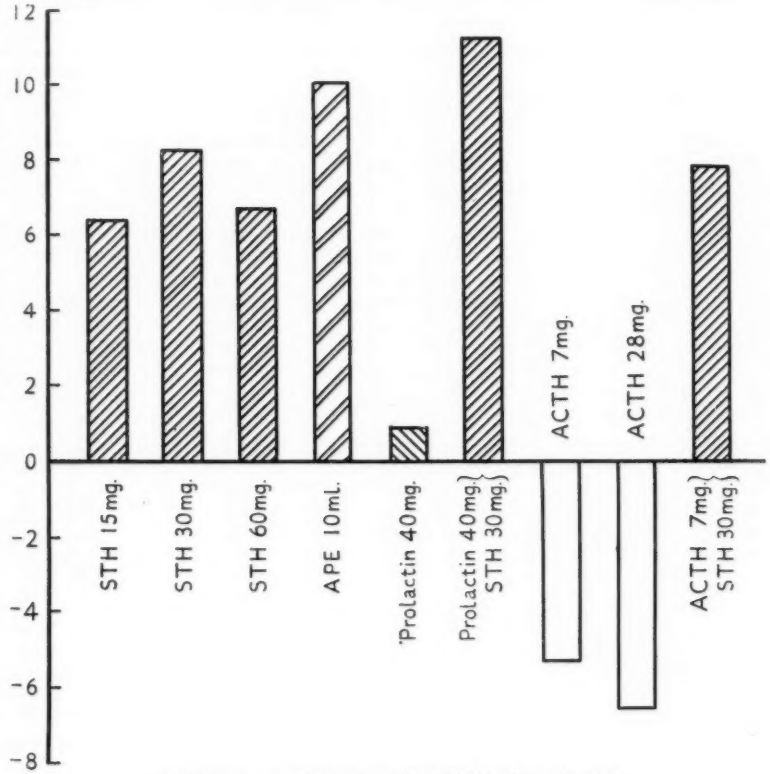
i. It is unwise to assume that determinations of changes in the prolactin content of the hypophysis, upon which the Meites-Turner theory was founded, give any indication of the changes in the rate of prolactin secretion. They should not be given too much weight in the present context, since they are rather difficult to interpret.

ii. Relatively low circulating levels of oestrogen activate the lactogenic and galactopoietic functions of the hypophysis, whereas high levels tend to inhibit lactation even in the presence of the ovary. In essence this is the so-called "double-threshold" theory of Folley & Malpress (1947, 1948b).

iii. "Lactogenic" doses of oestrogen may be deprived of their lactogenic properties by suitable doses of progesterone, the combination then functioning as a potent inhibitor of lactation (see, for example, Cowie *et al.* 1952); this is the inhibiting influence that operates during pregnancy.

iv. At parturition the rate of progesterone secretion decreases markedly before that of oestrogen (see Atkinson & Leathem (1946) for the mouse), removing the inhibition which is replaced by the "lactogenic" effect of oestrogen acting alone.

FIG. 3. EFFECT OF SINGLE INJECTIONS OF VARIOUS OX ANTERIOR PITUITARY PREPARATIONS ON THE MILK-YIELDS OF GROUPS OF COWS ON THREE FARMS (data from Cotes, Crichton, Folley & Young, 1949)



Ordinates : net change in milk-yield (% of initial yield)

Bars show the change in yield observed during the 2 days following the injection, expressed as a percentage of the initial yield, corrected for the change in yield for control cows

STH : somatotrophin
APE : unfractionated ox anterior pituitary extract
ACTH : adrenocorticotrophic hormone

The reader will also find the latest views of Meites (1954), on the hormonal control of lactogenesis, of interest.

3. Maintenance of Lactation: Galactopoiesis

The study of galactopoiesis—the experimental stimulation of lactation—is likely to throw light on the mechanism of the maintenance of lactation, since the hormonal mechanisms involved are probably closely related. Studies designed to determine the nature of the hormones responsible for the well-known galactopoietic activity of unfractionated extracts of ox anterior lobe were reviewed by Young (1947) in the Lactation number of this Bulletin and later by Folley & Young (1952).

a. Somatotrophin

In continuation of these studies, Cotes, Crichton, Folley & Young (1949), keeping in mind earlier observations suggesting that galactopoietic properties were bound up with diabetogenic activity, were prompted by the discovery that purified somatotrophin is diabetogenic (Cotes, Reid & Young, 1949) to examine this hormone for galactopoietic activity in cows. In accord with expectation, somatotrophin duly proved to be strongly galactopoietic in single-injection tests (see fig. 3). This discovery has since been confirmed in cows by Donker & Petersen (1951, 1952), Chung, Shaw & Gill (1953), Wrenn & Sykes (1953), Brumby & Hancock (1955) and Shaw, Chung & Bunding (1955), and in sheep by Jordan & Shaffhausen (1954). The galactopoietic activity of somatotrophin, which is of fundamental interest from the endocrinological standpoint, could also have important practical significance if it should prove, as suggested by preliminary experiments reported by Shaw (1955), that treatment with somatotrophin for a short period around parturition will raise the bovine lactation curve above the expected level for the whole of the ensuing lactation.

An interesting and important question is how somatotrophin exerts its galactopoietic action: does it merely increase the supply of milk precursors or does it directly regulate the metabolic activities of the milk-secreting cells? In an attempt to answer this question, Mary L. McNaught, in our laboratory, is studying the effects of somatotrophin in vitro on mammary gland slices from lactating rats. In presence of glucose, such slices show an active oxygen consumption ($-Q_{O_2}$, approx. 9.3) and a high R.Q. (approx. 1.5); this has been interpreted as evidence that glucose is utilized for, among other purposes, the net synthesis of fat from carbohydrate (Folley & French, 1949a). In preliminary experiments, addition to the incubation medium of a purified somatotrophin preparation (100 μg./ml.) increased $-Q_{O_2}$, R.Q. and the glucose uptake (McNaught, unpublished). This effect, which in many ways resembles that due to insulin (see below), seems to be essentially of a galactopoietic nature. It remains to be seen whether it can be substantiated as an inherent property of somatotrophin itself or whether it is due to a contaminant, say, intermedin (see Folley, 1955).

b. Prolactin

The earlier studies of Folley and Young (see Young, 1947; Folley & Young, 1952) suggested that the galactopoietic activity of prolactin was not very great. This was confirmed later by Cotes, Crichton, Folley & Young (1949) (see fig. 3), Biavati & Fiori (1950), Donker & Petersen (1952), and Wrenn & Sykes (1953).

c. Adrenocorticotrophin

Despite the fact that numerous studies by A. T. Cowie and others (see Folley, 1953, for review) show that the integrity of the pituitary-adrenal axis is necessary for maintenance of normal lactation, injection of ACTH exerts an inhibitory rather than a stimulatory action on lactation in the cow, as shown in fig. 3 (Cotes, Crichton, Folley & Young, 1949; Shaw, Hatziolos, Leffel, Chung, Gill & Gilbert, 1952; Flux, Folley & Rowland, 1954; Shaw *et al.* 1955). The mechanism of this action is not yet known.

For an account of all but the most recent experiments on maintenance of lactation in adrenalectomized rats, the reader should consult the review by Folley (1953) and also the paper by Cowie (1952) in which, inter alia, he shows that virtually complete maintenance of lactation after adrenalectomy in our rats could be achieved by treatment with the hormone combination, cortisone + deoxycorticosterone. Recently, Cowie & Tindal (1955) in preliminary experiments have studied the lactation-maintaining abilities of aldosterone, fluorohydrocortisone and chlorohydrocortisone in adrenalectomized rats of our colony. Chlorohydrocortisone, in a dose of only 100 μg. daily, gave virtually complete maintenance of lactation, though an equal daily dose of the fluoro compound or 50 μg./day of aldosterone were not so effective. The lactation-maintaining activities of a number of 11-deoxycorticoids in adrenalectomized lactating rats have recently been studied by Flux (1955) in our laboratory.

d. Pituitary-Thyroid Axis

The galactopoietic activity in cows of thyroid-active materials, known since the studies of Hertoghe (1896), was reviewed in this Bulletin by Young (1947) and since then by Blaxter, Reineke, Crampton & Petersen (1949) and by Blaxter (1952). An interesting recent development was the finding (Bailey, Bartlett & Folley, 1949) that crystalline L-thyroxine gave galactopoietic responses in cows, given by the oral route in doses (*ca* 70 mg./day) which, in view of the improved synthesis of L-thyroxine described by Chalmers, Dickson, Elks & Hems (1949), would enable one to replace iodinated casein by L-thyroxine for administration to cows, if this method of increasing milk-yield ever became a practical proposition. Incidentally, a large-scale study has shown that administration to cows of moderate doses of L-thyroxine for limited periods in each of three successive lactations has no significant ill-effects on their health and reproductive functions (Leech & Bailey, 1953). Surprisingly, L-triiodothyronine, though active subcutaneously, shows very little activity on the milk-yield or the heart rate of cows when it is given orally, probably because the rumen micro-organisms quickly learn how to inactivate it (Bartlett, Burt, Folley & Rowland, 1954). There is thus no prospect of using it in practice for increasing the milk-yield of cows.

e. Endocrine Pancreas

Since the mammary gland uses glucose for oxidative energy production, for lactose synthesis and, in the case of non-ruminants, for lipogenesis, it might be expected that insulin would play a part in the regulation of lactation through its control of the blood-sugar level and also perhaps peripherally through its action on the various pathways of glucose utilization. While the role of insulin in lactose biosynthesis is too obscure to merit discussion here, much is known about its effects on mammary lipogenesis, which are displayed markedly in vitro. Insulin, added to the incubation medium (substrate: acetate + glucose, or glucose), increases R.Q., $-Q_{O_2}$ and $-Q_{glucose}$ of mammary slices from lactating rats but not from rats at the end of pregnancy or after weaning (Balmain, French & Folley, 1950; Balmain & Folley, 1951). The inference that this means that insulin increases lipogenesis from acetate and glucose was substantiated by the demonstration (see Table I) that, if labelled substrates (^{14}C-acetate or ^{14}C-glucose + [*carboxy*-^{13}C-*methyl*-^{3}H] acetate) are used, the incorporation of both acetate and glucose carbon into the fatty acids of the slices is increased (Balmain, Folley & Glascock, 1952, 1954).

By contrast, udder slices from ruminants (sheep) show no response to insulin (Table I). The difference in this respect

TABLE I. EFFECT OF INSULIN, ADDED IN VITRO, ON LIPOGENESIS IN LACTATING MAMMARY GLAND SLICES INCUBATED WITH $C^3H_3{}^{13}COONa$ (0.02 M) + ^{14}C-GLUCOSE (0.3%)

(Results from Balmain, Folley & Glascock, 1954)

Source of tissue	Addition	Isotope content of Ca salts of mixed fatty acids after 3 hr.		
		^{14}C (counts/min./mg.C)	^{3}H (counts/min./mg. combustion H_2O)	^{13}C (atom-% excess)
Rat	None	381	95	0.054
	Insulin (1 i.u./ml.)	805	202	0.14
Sheep	None	22.5	501	0.373
	Insulin (1 i.u./ml.)	25.3	485	0.334

between mammary tissue from the rat and sheep may be connected with the fact that while rat mammary tissue, when confronted with a mixture of acetate + glucose, uses both for lipogenesis to an approximately equal extent, sheep udder slices use acetate almost exclusively (Balmain *et al.* 1954). This latter fact suggests that ruminant (sheep) udder tissue lacks the glycolytic pathway through which glucose is broken down to acetyl-coenzyme A before undergoing oxidation via the Krebs cycle or entering the lipogenic pathway. Experiments with ^{14}C-glucose in our laboratory have, however, shown that sheep udder tissue can oxidize glucose (Duncombe & Glascock, 1953), which prompted the present author and his colleagues (Balmain *et al.* 1954; Folley, 1954) to suggest that the so-called direct oxidation shunt (see Dickens, 1953) is an important pathway of carbohydrate oxidation in mammary tissue. Evidence substantiating this suggestion for the rat has since been provided by Glock & McLean (1954) and Abraham, Hirsch & Chaikoff (1954).

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THE POSTERIOR PITUITARY GLAND IN RELATION TO REPRODUCTION AND LACTATION

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In contrast to the large number of distinct pharmacological actions of posterior pituitary extracts, only one, namely the antidiuretic effect, has long been recognized to be of undoubted physiological significance (Waring & Landgrebe, 1950). This review will be concerned with evidence for the endocrine status of oxytocin. It is likely that progress in this somewhat neglected field will receive a stimulus from the recent synthesis of oxytocin by du Vigneaud, Ressler, Swan, Roberts, Katsoyannis & Gordon (1953). One result of the use of highly purified oxytocic and vasopressor-antidiuretic (antidiuretic hormone) polypeptides has been the demonstration that the latter possesses some intrinsic " oxytocic " activity, for example on the uterus (Popenoe, Pierce, du Vigneaud & van Dyke, 1952) and the mammary gland (Cross & van Dyke, 1953); but there is no evidence that antidiuretic hormone is involved in the control of reproductive functions.

1. Milk Ejection

A reflex process of milk ejection occurring in suckling or milking (" let-down " or the " draught ") has been described in many species, including the dog, cat, rabbit, goat, cattle, and man, and probably occurs in all mammals. In the rabbit, for example, it is characterized by a latent period of $\frac{1}{2}$–$1\frac{1}{2}$ min., followed by a rapid rise in intramammary pressure associated with ejection of milk from cannulated teats, a visible distension of unsuckled mammae and a fast transfer of milk to suckling young. The response subsides in a few minutes. There can be no doubt as to the functional importance of the reflex for, when it is inhibited by anaesthesia (Gaines, 1915; Cross & Harris, 1952; Cross, 1955b) or emotional disturbance (Ely & Petersen, 1941; Newton & Newton, 1948; Whittlestone, 1951; Cross, 1953a, 1955b), only a fraction of the normal milk-yield is obtained.

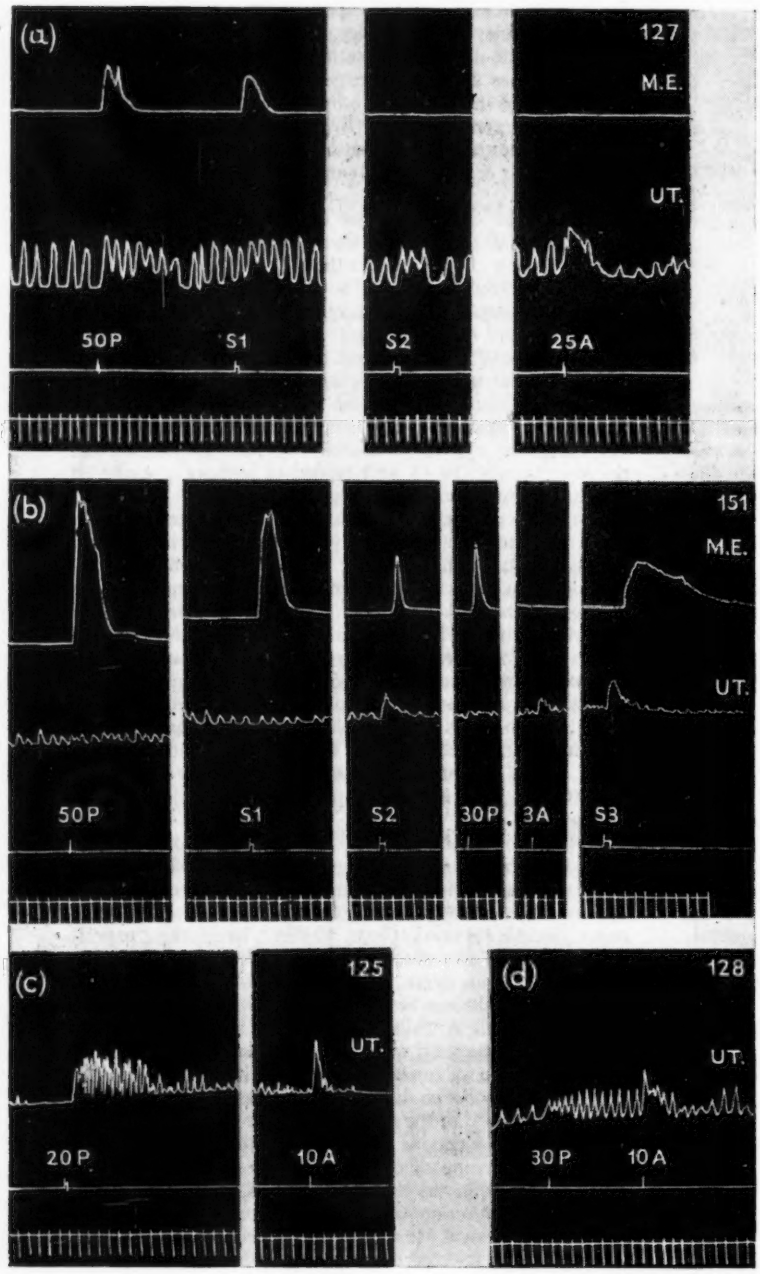
Belief in a simple spinal reflex mechanism could not be maintained in face of repeated failure to demonstrate any impairment of milk ejection following interruption of the mammary motor nerve supply. A neurohormonal reflex mechanism was then proposed (Ely & Petersen, 1941), with oxytocin from the posterior pituitary gland as the efferent link. Until quite recently this theory relied for its support on the similarity of the milk-ejecting effect of oxytocic extracts to the natural response, and on the enhanced milk-ejecting activity in perfused bovine udder preparations of jugular blood from cows that had been stimulated to let down their milk, as compared with control blood samples (see reviews by Petersen, 1948; Macauley, 1951; Folley, 1952; Silver, 1952). In the present decade direct confirmation of the posterior pituitary hypothesis has come from experiments involving electrical stimulation of the supraoptico-hypophyseal (SOH) system in goats (Andersson, 1951a, 1951b) and rabbits (Cross & Harris, 1952), and removal or denervation of the neurohypophysis in rabbits (Cross & Harris, 1952) and rats (Harris & Jacobsohn, 1952).

Perhaps the clearest picture of the physiological events underlying natural milk ejection can be seen in the rabbit (Cross, 1955c). In this species the reflex response in suckling can be duplicated by electrical stimulation of the infundibular stem or by injection of 0.05 i.u. of oxytocin. Either procedure enables normal removal of milk by the young from anaesthetized does. Electrical stimuli applied to the vicinity of the paraventricular nucleus have elicited milk ejection (fig. 1), in addition to stimulation of the supraoptic and median eminence regions, the infundibular stem and neural lobe (Cross, 1955a). In all cases the response to neurohypophyseal stimulation had the long latency (20–45 sec.) typical of humorally mediated effects. Stimulation was no longer effective in producing milk ejection after electrolytic lesions in the anterior median eminence had caused neurohypophyseal atrophy. These lesions did not affect nursing behaviour or prevent milk secretion, but the litters were unable to remove more than a fraction of the milk present in the mammary glands unless posterior pituitary replacement was given to the doe just before nursing (Cross & Harris, 1952). It would seem that oxytocin is the physiological milk-ejection hormone, since it is approximately six times more active than antidiuretic hormone as measured on the mammary gland of the lactating rabbit (Cross & van Dyke, 1953). Moreover the small release of antidiuretic hormone occurring in suckled does (Cross, 1951) is insufficient to exert any effect on milk ejection.

Further confirmation of the essential role of posterior pituitary hormone in promoting removal of milk from the mammary glands has come from a study of the mechanisms of emotional disturbance of milk ejection in rabbits suckled while under forcible restraint (Cross, 1955b). In 35 of 42 experiments the milk-yield obtained by the young was reduced by 20–100%. Kymographic records of milk-ejection responses indicated a parallelism between the size of the reflex response and the amount of milk withdrawn. Thus normal milk removal was associated with a reflex response corresponding to about 0.05 i.u. of injected oxytocin, half the normal milk-yield with a response equivalent to 0.005–0.01 i.u. of oxytocin, and less than 15% of the milk-yield with complete absence of any reflex milk ejection. Emotional inhibition of oxytocin release was accompanied in a few cases by a peripheral inhibitory effect in the mammary glands, as shown by the failure of an injection of 0.05 i.u. of oxytocin to restore normal milk removal. In such cases the recorded milk-ejection response to the injected oxytocin was reduced in size by an

FIG. 1. EFFECT OF HYPOTHALAMIC STIMULATION ON MILK EJECTION AND UTERINE MOTILITY

M.E.: kymographic record of milk-ejection responses from cannulated teats
UT.: kymographic tracing of uterine activity recorded by means of a small intra-uterine balloon
A, P: points of intravenous injection of adrenaline and Pitocin in doses of μg. and milliunits, respectively
S: point of electrical stimulation (1.0 v a.c., sine wave, 50 cyc./sec.)
Time signal registers 30-sec. intervals
Numerals in top right-hand corners refer to the serial numbers of the experimental animals



a: records in rabbit, six days post partum: no suckling had taken place; animal in oestrus (prompt lordosis when placed with buck); head was held in stereotaxic instrument; Nembutal anaesthesia was given.

50P: 50 milliunits (0.05 i.u.) Pitocin injected

S1: 10-sec. stimulation of supraoptico-hypophyseal (SOH) tract in infundibular stem

S2: 15-sec. stimulation in right lateral hypothalamic area (pupil dilatation and hyperpnoea were observed)

25A: 25 μg. adrenaline injected

Note "spontaneous" uterine contractions, and similarity of the effects on the uterus and mammary gland of SOH stimulation and injection of Pitocin. Stimulation of the hypothalamic sympathetic area gave no milk ejection but evoked a uterine response similar to that following adrenaline. The response to adrenaline is followed by a partial inhibition of the rhythmic uterine activity (cf. the "immediate biphasic reaction" discussed by Ferguson, 1941, and fig. 1c and fig. 1d below).

b: records in rabbit, on 41st day of lactation: animal in lactation oestrus (prompt lordosis with buck, but uterus involuted); head was held in stereotaxic instrument; Nembutal anaesthesia was given.

50P: 50 milliunits (0.05 i.u.) Pitocin injected

S1: 10-sec. stimulation of SOH tract in infundibular stem

S2: 15-sec. stimulation of dorsal hypothalamus just lateral to left paraventricular nucleus (magnocellular portion)

30P: 30 milliunits (0.03 i.u.) Pitocin injected

3A: 3 μg. adrenaline injected

S3: 20-sec. stimulation at same site as S2

Note that the uterus is unresponsive to endogenous posterior pituitary hormone or injected Pitocin but responds to injection of adrenaline or hypothalamic stimulation (S2 and S3). This stimulation in the vicinity of the paraventricular nucleus also caused a delayed milk-ejection response indicating release of oxytocin.

c, d: records of uterine activity from two unanaesthetized rabbits in oestrus, with uterine fistulae.

20P: 20 milliunits (0.02 i.u.) Pitocin injected

10A: 10 μg. adrenaline injected

30P: 30 milliunits (0.03 i.u.) Pitocin injected

10A: 10 μg. adrenaline injected

Compare the uterine response to Pitocin and adrenaline with figs. 1a and 1b.

amount similar to that produced by an intravenous dose of 1 μg. of adrenaline given just before 0.05 i.u. of oxytocin. It is extremely probable that the peripheral block disclosed by these experiments involves a mammary vasoconstriction due to activation of sympathetico-adrenal pathways from the hypothalamus (Cross, 1953a, 1955a).

It should perhaps be stressed that confirmatory evidence from other species is necessary before the mammary function of the neurohypophysis will be as generally acceptable as the renal function. Previous work involving experimental interferences in the hypothalamo-hypophyseal region has led to equivocal results with regard to lactation, and in no case was the efficiency of milk removal specially studied (see discussion by Macauley, 1951). The usual criterion of lactational performance was survival or death of the young. Failure to rear litters cannot be attributed to impairment of milk ejection in the absence of successful posterior pituitary replacement therapy, since there can be no guarantee that milk secretion is adequate. On the other hand, survival of the young does not necessarily imply a fully functional neurohypophyseal milk-ejection mechanism. Some milk may be extractable from lactating mammary glands in the absence of the milk-ejection reflex. Non-hormonal mechanisms such as mechanically induced contraction of mammary myoepithelium (Cross, 1954) possibly play a part in this. These considerations may apply to the two cats (Fisher, Magoun & Ranson, 1938) and one woman (Dandy, 1940), where a superficially normal lactation occurred despite the existence of a chronic diabetes insipidus.

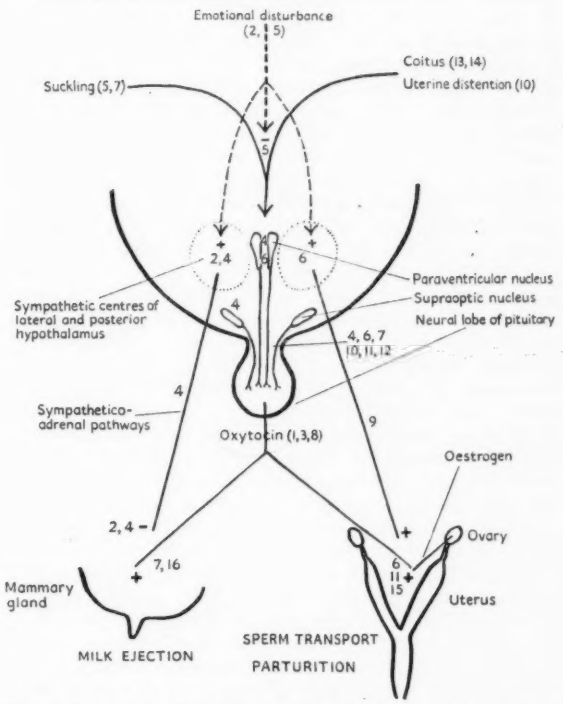
2. Parturition

The increased rhythmic contractions of the oestrous, oestrogenized or post-parturient uterus in vivo following injection of posterior pituitary extracts (Reynolds, 1930; Ferguson, 1941; Harris, 1947) or electrical stimulation of the neurohypophysis (Harterius & Ferguson, 1938; Ferguson, 1941; Harris, 1947, 1948a), suggest the possibility of a physiological role for the gland in parturition. The ability of oxytocic extracts to induce labour at term is, of course, well known. Franklin & Winstone (1954) have observed that the course of parturition thus initiated is indistinguishable from natural labour in the rabbit. That oxytocin is secreted into the blood at parturition is indicated by the occurrence of milk ejection in lactating human subjects (Gunther, 1948) and by the appearance of an oxytocic substance in the blood (Bell & Morris, 1934; Bell & Robson, 1935) and in the urine (Cockrill, Miller & Kurzrok, 1934).

Probably the most convincing evidence so far for the reflex activation of the posterior pituitary gland in labour is that of Ferguson (1941). He found that uterine or cervical dilatation in post-parturient rabbits resulted in a " slow augmentation reaction " in the uterus, resembling that produced by injection of oxytocic extract. This response was abolished by electrolytic destruction of the pituitary stalk. He also described an " immediate biphasic reaction " produced by dilatation of the vagina, which was similar to the effect of injecting adrenaline. This response was not abolished by pituitary stalk destruction.

Evidence from experimental removal of the pituitary, its neural lobe, or interruption of the SOH tract in the hypothalamus, is conflicting (see reviews by Harris, 1948b; Waring & Landgrebe, 1950). Some have recorded apparently normal labour; others observed prolonged labour with death of the young in some cases. It seems unlikely that these discordant results can be explained solely by the survival or otherwise of neurohypophyseal remnants following operation, for normal parturition occurred in some individuals with a diabetes insipidus (Dandy, 1940; Dey, Fisher & Ranson, 1941). Unlike the mammary gland, the uterus receives a functional motor nerve supply (Reynolds, 1949; Marshall & Moir, 1952). Contractions of the organ may be induced by stimulation of the sympathetic centres of the hypothalamus as well as by stimulation of the neurohypophysis (figs. 1, 2). Moreover, in parturition, delivery is certainly assisted by contractions of the abdominal muscles. It seems likely, therefore, that several mechanisms operate in labour, and that the relative importance of posterior pituitary secretion may vary between species and between individuals.

FIG. 2. DIAGRAM OF NERVOUS AND HORMONAL REGULATION OF MILK EJECTION AND UTERINE MOTILITY IN THE RABBIT



The numbers on the figure relate to references giving experimental evidence as follows: 1: Cross (1951); 2: Cross (1953a); 3: Cross (1953b); 4: Cross (1955a); 5: Cross (1955b); 6: Cross (unpublished observations, see fig. 1); 7: Cross & Harris (1952); 8: Cross & van Dyke (1953); 9: Cushny (1906); 10: Ferguson (1941); 11: Harris (1947); 12: Harterius & Ferguson (1938); 13: Heape (1898); 14: Krehbiel & Carstens (1939); 15: Reynolds (1930); 16: Turner & Cooper (1941)

It should be noted that there is so far no direct evidence that in the rabbit coitus stimulates the neurohypophysis, or that the neurohypophysis is essential for normal parturition (see text). The afferent pathways by which sensory impulses from the mammary gland and reproductive tract reach the hypothalamus are unknown.

3. Sperm Transport

a. In the Female

That post-coital uterine contractions might accelerate the ascent of sperm has long been suspected (Heape, 1898; Parker, 1931; Krehbiel & Carstens, 1939). It is well known that coitus stimulates the release of gonadotrophic hormone from the anterior pituitary gland in certain species (rabbit, cat and ferret). This fact, together with the dramatic increase in uterine activity following stimulation of the SOH tract by the remote control technique in conscious oestrogenized rabbits, led Harris (1947) to suggest that neural activation of the posterior pituitary gland might be involved in the mechanism of sperm transport. A fair amount of circumstantial evidence has accumulated in support of this view. Secretion of posterior pituitary hormone, as indicated by ejection of milk from the breast, has been observed during coitus in the human subject (Pickles, 1953; Harris & Pickles, 1953); and a similar effect occurs in animals (Hammond, 1936; Andersson, 1951a).

Salisbury & VanDemark (1952) claimed that posterior pituitary mediation of sperm transport has been established for the cow on the following grounds. (i) A few sperm may be recovered from the Fallopian tube in as short a time as 2¼ min. after insemination of live or dead sperm into the cervix (VanDemark & Moeller, 1951). (ii) A rise of intramammary pressure ($>$15 mm. of water) occurred 10–30 sec. after stimulation of the cervix (as in technique of artificial insemination) or of the vulva in lactating animals (Hays & VanDemark, 1953a). (iii) Strong uterine contractions were induced in oestrous or post-oestrous cows by various mating stimuli, including the sight of the bull (VanDemark & Hays, 1952). Intravenous injection of 15 i.u. of oxytocin was stated to produce a similar response, whereas 2 mg. of adrenaline caused inhibition of uterine activity after an initial tetanic contraction. (iv) Further experiments on isolated perfused bovine uteri were mentioned, in which oxytocin caused increased activity of the organ and transport of inseminated sperm from the cervix to the ovarian end of the oviduct in less than 5 min. While all this evidence is suggestive, conclusive proof of the hypothesis must await the demonstration that removal or destruction of the neurohypophysis impedes the ascent of sperm, and that denervation of the uterus does not. The short latency ($<$ 10 sec.) of the uterine response to mating observed by VanDemark & Hays (1952) might suggest the participation of nervous factors, since the milk-ejection reflex, which probably involves release of about 1 i.u. of oxytocin in the cow, has a latent period nearer 1 min. It takes about 18 sec. for blood to circulate from the jugular vein to the udder (Vercruysse, Peeters & Coussens, 1950). Moreover, the published records of these uterine responses to mating stimuli seem to bear as much resemblance to those resulting from injection of 2 mg. of adrenaline as to those following injection of 15 i.u. of oxytocin (Hays & VanDemark, 1953b).

It is to be hoped that further data on the mechanism of sperm transport may also throw light on its relation—if any—to fertility, for this is by no means clear as yet. In this connexion it is of interest that removal or denervation of the posterior pituitary gland is not incompatible with conception and pregnancy (Smith, 1932; Dempsey & Uotila, 1940; Cross & Harris, 1952; Dandy, 1940; Harris & Jacobsohn, 1952).

b. In the Male

The way sperm migrate from the seminiferous tubules to the cauda epididymis is obscure, although seminal emission seems to be adequately explained in terms of autonomic reflexes. The evidence for oxytocin release in coitus in the female (see above) suggests a possible parallel in the male. Microscopic examination of the male organs of the rabbit, using an abdominal chamber technique (Cross, unpublished), failed to show any effect of intravenous oxytocin on the accessory glands, but there appeared to be some stimulation of the contractile mechanisms of the testis and epididymis. Peristaltic and pendular movements were seen in the tubules of the head and body of the epididymis, and segmentation contractions in the tubules of smaller calibre in the tail. In the testis slow rhythmic contractions of the tunica propria and albuginea were observed alternately on the two sides, so that contraction on one side was associated with relaxation and increasing convexity on the other. Plethysmograph experiments showed that these contractions involved no change in volume of the testis as a whole, whereas injection of 0.5 μg. of adrenaline caused a visible vasoconstriction and a transient shrinkage in volume, amounting to about 0.7%. Much further work is required to evaluate the significance of these observations.

4. Neural Control of Oxytocin Secretion

The evidence seems fairly conclusive that oxytocin plays an essential part in milk ejection, and very suggestive that it is also concerned in parturition and sperm transport. It is therefore of agricultural and obstetric interest that reflex release of oxytocin may be inhibited in emotional disturbance (Cross, 1955b; see also fig. 2). From the more academic view-point a better understanding is required of the secretomotor nerve supply governing oxytocin formation and release. The minimal or absent secretion of antidiuretic hormone following suckling, i.e. the natural stimulus to oxytocin secretion, in the cow (Peeters & Coussens, 1950), rabbit (Cross, 1951), and dog (Kalliala, Karvonen & Leppänen, 1952), argues for some degree of independence in the control of the two neurohypophyseal hormones. In the rabbit, too, the ratio oxytocin: antidiuretic hormone released following suckling (approx. 100:1) is about 20 times greater than that following electrical stimulation of the SOH tract (approx. 5:1, Harris, 1948a). This evidence is difficult to reconcile with a unitary conception of posterior pituitary hormone. Now it would appear that stimulation of the region of the paraventricular nucleus causes release of oxytocin (Cross, 1955a; see also fig. 1) but not of antidiuretic hormone (Harris, 1947), whereas stimulation of the infundibular stem causes secretion of both hormones. The suggestion that the paraventricular nucleus is specifically concerned with oxytocin secretion (Olivecrona, 1954) clearly merits further study.

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ROLE OF THE ADRENAL CORTEX IN REPRODUCTION

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1 Sexual dimorphism of the adrenal
2 Action of sex hormones
3 The X zone
4 The human foetal or transient cortex
5 The adrenogenital syndrome
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1. Sexual Dimorphism of the Adrenal

The gonads and the adrenal cortex perform distinct physiological roles, yet they maintain a close interdependence, the nature of which still perplexes us (Parkes, 1945; Burrows, 1949; Courrier, Baclesse & Marois, 1953; Zuckerman, 1953). The first challenge is given, with the answer still to find, by the post-pubertal dimorphism of the adrenal (Table I). The mouse, characteristic of many eutherian species, has, in the male, an adrenal weight between 65% and 75% of that of the female; the rat shows similar differences (Tables II, III). In all species examined, the differences in weight are due to variations in cortical size (Parkes, 1945; Chester Jones, 1948; Hartman & Brownell, 1949). How far this reflects differences in cortical function and activity is not known. Moreover, in the golden hamster, the weight of the adrenal of the non-hibernating female is 78.8% of that of the male (Table IV).

TABLE I. BODY AND ADRENAL WEIGHTS OF TWO STRAINS OF MICE AT DIFFERENT AGES, ILLUSTRATING THE ADRENAL DIMORPHISM CHARACTERISTIC OF MANY MAMMALS (Chester Jones, unpublished work)

	Age (months)	Number of animals in each group	Body-weight (g.)	Adrenal weight (mg.)
	Strain: Strong A			
Adult males	2–4	45	23.00 ± 1.12*	2.58 ± 0.03*
Adult males	6–8	18	28.72 ± 0.59	2.83 ± 0.07
Unmated females	4	17	23.20 ± 1.38	3.88 ± 0.10
Parous females	6–7	24	24.62 ± 0.34	3.63 ± 0.18
	Strain: Bagg C Albino			
Adult males	4	28	26.2 ± 1.23	3.50 ± 0.09
Adult males	8	27	25.8 ± 1.54	3.94 ± 0.11
Unmated females	4.5	28	20.26 ± 1.58	5.19 ± 0.11
Parous females	8	15	25.22 ± 0.69	6.47 ± 0.20

* Means ± standard error.

TABLE II. EFFECT OF INJECTION OF OESTRADIOL BENZOATE (OB) AND OF TESTOSTERONE PROPIONATE (TP) ON ADRENAL WEIGHT OF NORMAL AND CASTRATED MALE RATS

The rats were 30 days old at the start of the experiment and were injected daily for 45 days (cf. the data of Greep & Chester Jones (1950) shown graphically in their fig. 31 as adrenal weight in mg./100 g. of body-weight against dose).

	Number of animals in each group	Body-weight (g.)	Adrenal weight (mg.)	Adrenal weight (mg./100 g. body-weight)
Normal males:				
controls (30 days old)	11	75.4± 4.83*	15.25±0.60*	22.38±0.83*
controls (75 days old)	19	220.1± 6.55	36.63±1.61	17.05±1.00
OB (0.0033 μg.)†	9	215.3± 8.13	38.52±1.87	18.07±1.11
OB (0.0166 μg.)	11	231.0± 4.82	34.95±1.56	15.12±0.49
OB (0.166 μg.)	7	208.0± 6.22	51.80±5.42	25.31±3.05
OB (0.332 μg.)	9	189.2± 7.43	54.82±3.06	29.19±1.40
OB (1.66 μg.)	10	162.5± 4.11	47.49±2.36	29.18±0.98
TP (10 μg.)	7	228.8±11.30	34.45±2.20	15.10±0.73
TP (20 μg.)	10	256.2± 5.71	38.00±1.61	14.82±0.47
TP (50 μg.)	9	226.7± 4.95	31.05±0.75	13.74±0.42
TP (100 μg.)	8	197.1± 6.03	28.80±1.14	14.64±0.50
Castrated males:				
untreated	11	195.4± 3.09	41.18±1.51	21.60±1.36
OB (0.0033 μg.)	7	208.2± 5.65	38.29±3.79	18.40±1.57
OB (0.0166 μg.)	9	208.0± 3.47	42.48±2.88	20.42±1.30
OB (0.083 μg.)	10	202.8± 4.17	56.84±4.12	28.83±2.40
OB (0.166 μg.)	12	187.0± 7.79	58.40±2.89	31.86±2.09
OB (0.498 μg.)	9	154.7± 5.00	57.65±4.10	36.66±2.30
TP (10 μg.)	9	199.8± 6.94	30.14±2.31	14.51±0.75
TP (50 μg.)	8	196.6± 5.99	31.10±1.55	15.79±0.42

* Means ± standard error.

† 0.166 μg. ≡ 1 rat unit, in the notation of Greep & Chester Jones (1950).

2. Action of Sex Hormones

In two recent studies the gross effects of sex hormones on the adrenal cortex have been reinvestigated (Greep & Chester Jones, 1950; Carter, 1954; see also Tables II, III (i), (ii) of this paper). Sex hormones may well act directly on the adrenal cortex, but nevertheless the pituitary is probably the keystone of the changes. The anterior lobe of the pituitary can be supposed to have a fair degree of constancy of cell number. This, coupled with the presence of only three main types of cell yet several hormones, carries with it the possibility that increased secretion (with or without increased storage) of one hormone alters directly (Friedgood, 1946) or reciprocally (Selye, 1951) the secretion of other hormones. Thus, in addition to the " pituitary shift " of Selye, we must envisage the possibility of total increased secretion of many or all adenohypophyseal hormones (Zuckerman, 1953). When considering the adrenal-gonad relationship in its simplest terms, it can be imagined that there is an interplay between adrenocorticotrophic hormone (ACTH), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) storage and secretion. If the data of Tables II and III are taken together with those of Greep & Chester Jones (1950) on pituitary secretion and storage of gonadotrophins, then a possible explanation of the results can be postulated. Spaying results in the storage of FSH and LH, with unchanged pituitary weight, and this may prevent

TABLE III. EFFECT OF INJECTION OF OESTROGENS AND OF TESTOSTERONE INTO NORMAL AND SPAYED FEMALE RATS

(i) Normal and spayed female rats, aged 30 days at start of experiment, injected daily for 45 days with either oestradiol benzoate (OB) or testosterone propionate (TP) (cf. the data of Greep & Chester Jones (1950) shown graphically in their fig. 31 as adrenal weight in mg./100 g. of body-weight against dose). (ii) Normal female rats injected daily with 100 μg. of oestradiol dipropionate (ODP) for 22 days, and females spayed for 63 days and then injected for 12 days with 10 μg. of ODP (Carter, 1954).

	Number of animals in each group	Body-weight (g.)	Adrenal weight (mg.)	Adrenal weight (mg./100 g. body-weight)
(i) Normal females:				
controls (30 days old)	10	54.4±1.73*	13.79±0.95*	25.20±1.30*
controls (75 days old)	23	164.5±3.04	48.56±2.08	29.57±1.07
OB (0.0166 μg.)†	15	168.0±2.37	49.30±1.97	29.42±1.19
OB (0.166 μg.)	16	178.6±4.29	55.08±2.71	30.60±1.36
OB (0.332 μg.)	11	160.0±3.68	46.64±1.85	29.15±1.12
OB (0.498 μg.)	12	156.0±4.78	50.30±1.34	32.35±1.29
OB (1.66 μg.)	13	127.7±2.42	45.01±0.58	35.33±1.78
OB (4.98 μg.)	13	132.7±2.98	44.97±2.10	33.74±1.12
TP (1 μg.)	8	160.0±3.83	45.33±1.26	28.43±1.10
TP (3 μg.)	11	157.6±2.64	40.66±1.27	25.78±0.72
TP (10 μg.)	11	148.8±4.58	36.51±1.69	24.67±1.26
TP (20 μg.)	10	154.6±3.06	37.68±2.78	24.24±1.55
TP (50 μg.)	9	173.9±3.60	32.93±1.22	18.97±0.80
TP (100 μg.)	11	199.7±6.36	35.69±1.60	17.85±0.62
TP (500 μg.)	14	166.7±3.80	30.41±1.06	18.24±0.57
Spayed females: untreated	13	173.0±4.86	43.26±1.34	25.06±0.70
OB (0.166 μg.)	11	173.6±5.38	45.09±2.68	26.15±1.66
OB (0.830 μg.)	10	136.9±5.22	54.60±2.21	40.31±2.00
TP (10 μg.)	10	177.9±4.98	38.27±2.10	21.53±1.11
TP (50 μg.)	13	195.7±4.71	32.46±1.36	16.64±0.62
(ii) Normal females:				
controls (56–77 days old)	12	169.8±5.33	63.2 ±1.98	34.2 ±1.04
ODP (100 μg.)	9	161.1±4.77	47.3 ±1.45	29.6 ±1.24
sham-operated controls (182–210 days old)	14	187.0±1.91	59.4 ±1.56	31.6 ±0.74
ODP (10 μg.)	15	183.6±2.47	54.3 ±6.16	29.5 ±0.33
spayed females (182–210 days old)	13	227.3±4.36	48.8 ±1.76	21.4 ±0.44
ODP (10 μg.)	15	205.4±2.30	55.6 ±1.25	26.8 ±0.66

* Means ± standard error.
† 0.166 μg. ≡ 1 rat unit, in the notation of Greep & Chester Jones (1950).

some cells from manufacturing ACTH, so that there is a diminished secretion of ACTH. This is reflected in the narrow zona fasciculata of spayed females and in the fall in weight of the gland. On the other hand, in the male, castration is accompanied by increased storage of FSH and LH and also by increase in pituitary size. It may be that here gonadotrophin is stored by enlargement of cells only, and that no cells are taken away from ACTH production, so that the zona fasciculata of the castrated male is well maintained. Furthermore, the pituitary of the castrate secretes both LH and FSH copiously, while the spayed female pituitary secretes relatively much less. The thickening of the cortex of the castrate seems to be due to an increase in the inner region of the zona fasciculata, and this conceivably may be due to the increased secretion of LH. It is possible that, just as ACTH can have two or more facets in cell growth and in hormone secretion, so too can gonadotrophin exercise an influence on growth and maintenance of inner fasciculate cells without biological indication of hormone activity.

The injection of testosterone results in a diminution of adrenocortical size (despite some experimental evidence of its cortex-maintaining properties in the hypophysectomized animal, though this is not an unequivocal finding; see, for example, Zizine *et al.* 1950; Rennels *et al.* 1953). Testosterone reduces the size of the pituitary, favours FSH storage and lessens LH secretion. This in turn may be reflected in a decrease in size of the inner zona fasciculata; further, the increased storage of FSH could lead to a diminished secretion of ACTH, giving, overall, a smaller cortex. Oestrogens, on the other hand, seem to act by aiding the secretion not only of gonadotrophins but also of ACTH (Mandl & Zuckerman, 1952; Gemzell, 1952). It is possible that, in normal males, injected oestrogen liberates the stored FSH and allows more cells to produce ACTH, and that the consequent adrenal enlargement is aided by the reduction of testicular androgen, thus allowing thickening of the inner zona fasciculata. A similar pattern, *mutatis mutandis*, may be true of gonadectomized animals injected with oestrogens.

In normal females only high doses of oestrogens are effective in reducing adrenal weight (Table III (i), (ii)). It seems that the female shows a variety of reactions to exogenous oestrogens; doses over short periods can stimulate general pituitary secretion and allow adrenal enlargement, or interfere hardly at all with the mechanisms already operated by endogenous oestrogens, or, in high doses over long periods, the hormone may have a pharmacological effect of depression of pituitary secretion and may, in extreme cases, cause tumour formation.

The golden hamster presents an unexplained paradox (Table IV). It is doubtful whether spaying has much effect on adrenal size (cf. Keyes, 1949; Wexler, 1952). Castration tends to result in adrenal weight decrease, and exogenous testosterone unequivocally causes an increase in adrenal weight in both males and females. The very early maturity of the male hamster, at about 16 days of age, must be accompanied by significant gonadotrophin secretion in an immature soma. The basic ACTH–FSH–LH pattern may be thereby set differently and it is interesting to note that there is no sex difference in pituitary weight in the hamster as there is in the rat. In the hamster testosterone in moderate doses may effect the release and increased secretion of ACTH, a result seen in the rat only with very high amounts (Selye, 1941).

3. The X Zone

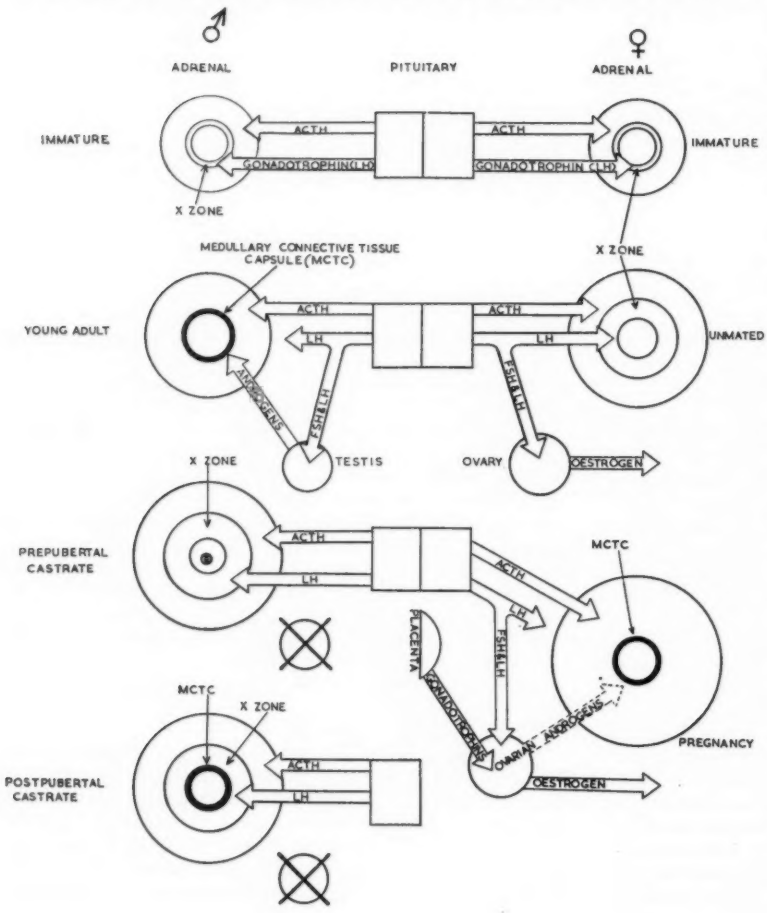
One of the most striking histological entities in the adrenal cortex, typically represented in the mouse, is the X zone, which comprises cells with acidophilic cytoplasm and prominent basophilic nuclei surrounding the medulla. The history of the zone is illustrated in fig. 1 and Plate V, figs. A–E. In the male the X zone collapses by the direct action of the androgens produced concomitantly with maturity (Deanesly &

TABLE IV. EFFECTS OF GONADECTOMY AND TESTOSTERONE PROPIONATE (TP) INJECTION ON ADENO-HYPOPHYSEAL AND ADRENAL WEIGHTS OF THE GOLDEN HAMSTER AGED ABOUT THREE MONTHS
(From Holmes, unpublished work)

	Number of animals in each group	Body-weight (g.)	Adrenal weight		Anterior pituitary weight	
			(mg.)	(mg./100 g. body-weight)	(mg.)	(mg./100 g. body-weight)
Unmated females . . .	6	56.1 ± 2.73*	8.66 ± 1.04*	15.46 ± 0.20*	1.75 ± 0.19*	3.18 ± 0.12*
Spayed 21 days earlier . .	6	74.1 ± 6.37	9.93 ± 1.21	13.46 ± 0.82	2.84 ± 0.08	3.83 ± 0.42
Spayed and injected with TP (2.0 mg./day) for 21 days . .	4	60.7 ± 1.71	13.80 ± 0.87	22.80 ± 1.97	2.26 ± 0.20	3.72 ± 0.17
Males	6	59.0 ± 2.00	11.00 ± 0.46	18.71 ± 0.43	1.64 ± 0.09	2.77 ± 0.04
Castrated 21 days earlier . .	6	82.2 ± 7.03	11.82 ± 1.22	13.09 ± 0.25	3.39 ± 0.09	4.27 ± 0.41
Castrated and injected with TP (2.0 mg./day) for 21 days . .	4	85.5 ± 3.80	19.87 ± 1.38	23.42 ± 2.42	2.39 ± 0.14	2.82 ± 0.91

* Means ± standard error.

FIG. 1. DIAGRAM ILLUSTRATING THE VARIOUS CHANGES IN THE ADRENAL CORTEX THROUGHOUT THE LIFE OF THE MOUSE



Parkes, 1937; Chester Jones, 1949a). The young adult male aged from 40 days onwards has no adrenal X zone, but a connective tissue capsule surrounds the medulla (Plate V, fig. D). Castration before puberty removes endogenous androgens and enhances gonadotrophin secretion, and the X zone continues to develop. In the unmated female the X zone continues to enlarge up to puberty, at which time it may occupy 50% of the cortex and can remain undegenerated for up to three months (Plate V, fig. A). Gradually in middle life the zone collapses, perhaps under the influence of small amounts of ovarian androgens (Chester Jones, 1949b, 1952). In first pregnancy, during days 7 to 12, the X zone disappears rapidly because of ovarian androgens produced under the influence of placental gonadotrophin (Chester Jones, 1952). Post-pubertal castration of the adult male mouse gives a very clear example of the capacity of gonadotrophins to act upon the inner zona fasciculata, thus evoking a wide secondary X zone (Plate V, fig. E). This demonstrates a potentiality in one species which may be present in others, as we have supposed in the case of the rat mentioned above. No function of the X zone is known. It has been thought to produce androgens (Grollman, 1936), but there is no evidence for this (Gersh & Grollman, 1939; Howard & Gengradom, 1940; Chester Jones, 1949a, 1949b, 1950).

4. The Human Foetal or Transient Cortex

The problem of the human foetal adrenal cortex (Plate V, fig. F) is bound up with the general problem of the possibility of

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(FIGS. A—H)

I. Chester Jones

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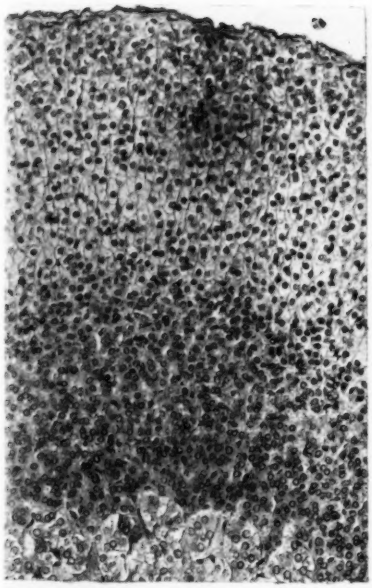


FIG. A

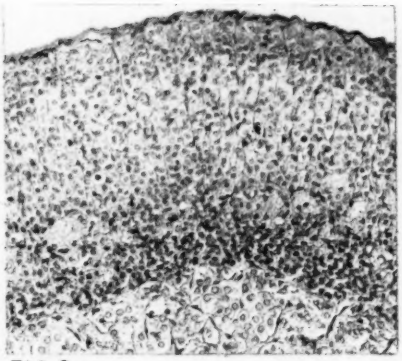


FIG. B

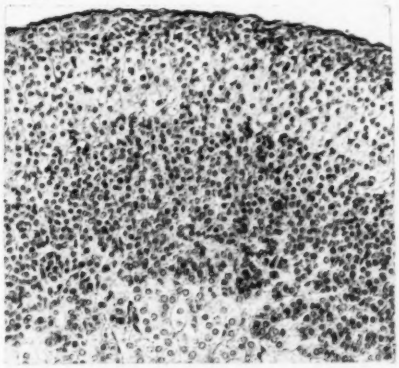


FIG. C

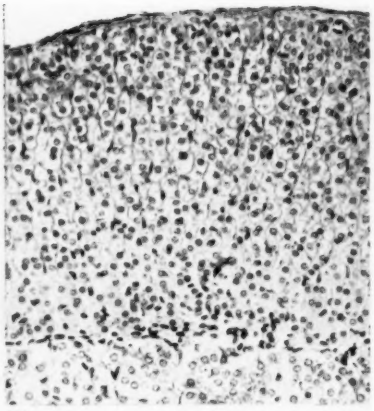


FIG. D

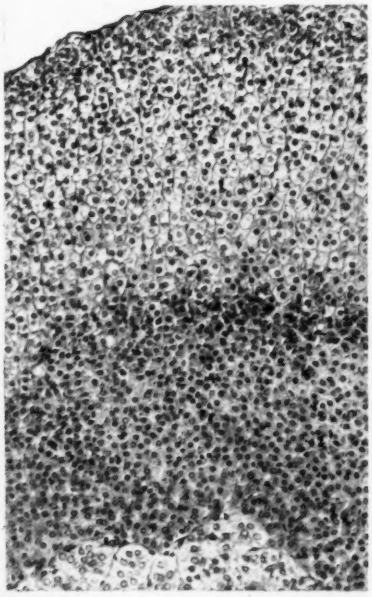


FIG. E

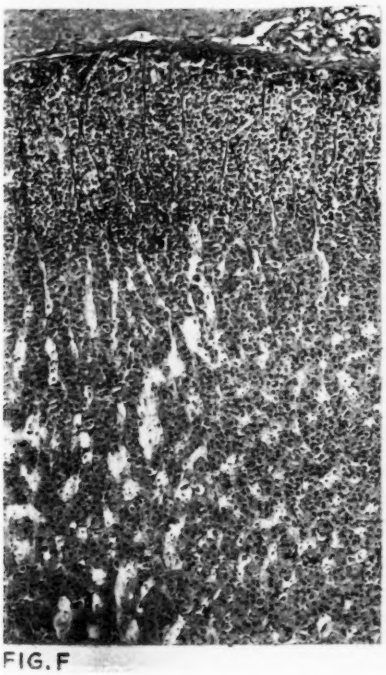


FIG. F

FIG. H

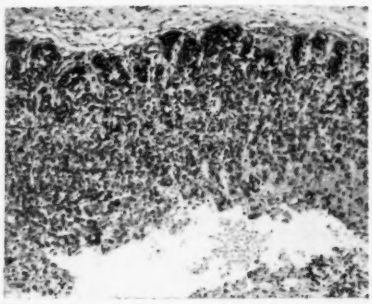


FIG. G

PLATE V. THE X ZONE IN THE MOUSE, AND IN THE HUMAN FOETAL CORTEX

(Each photomicrograph shows a portion of a paraffin wax section of adrenals fixed in either Bouin or Zenker-formol and stained in haematoxylin and eosin. We acknowledge with thanks the permission of the editor of *Endocrinology* to reproduce figs. A, B, C and E)

FIG. A. Unmated female mouse. The wide X zone lies against the medulla which is at the bottom of the photomicrograph. No zona reticularis is evident

(magnification × 180)

FIG. B. Hypophysectomized unmated female mouse. The X zone and zona fasciculata are degenerating; the zona glomerulosa is not

(magnification × 180)

FIG. C. Hypophysectomized unmated female mouse injected with pituitary gonadotrophin (principally LH). The X zone is well maintained, the zona fasciculata degenerating in the absence of ACTH, and the zona glomerulosa normal in appearance

(magnification × 180)

FIG. D. Young adult male mouse. There is no X zone, but a medullary connective tissue capsule surrounds the medulla

(magnification × 180)

FIG. E. Adult male mouse two months after post-pubertal castration. The wide secondary X zone has appeared through the action of pituitary gonadotrophin on the inner zona fasciculata cells

(magnification × 180)

FIG. F. Full-term baby. The adrenal shows the narrow rim of permanent cortex and, underneath, a portion of the extensive foetal cortex

(magnification × 85)

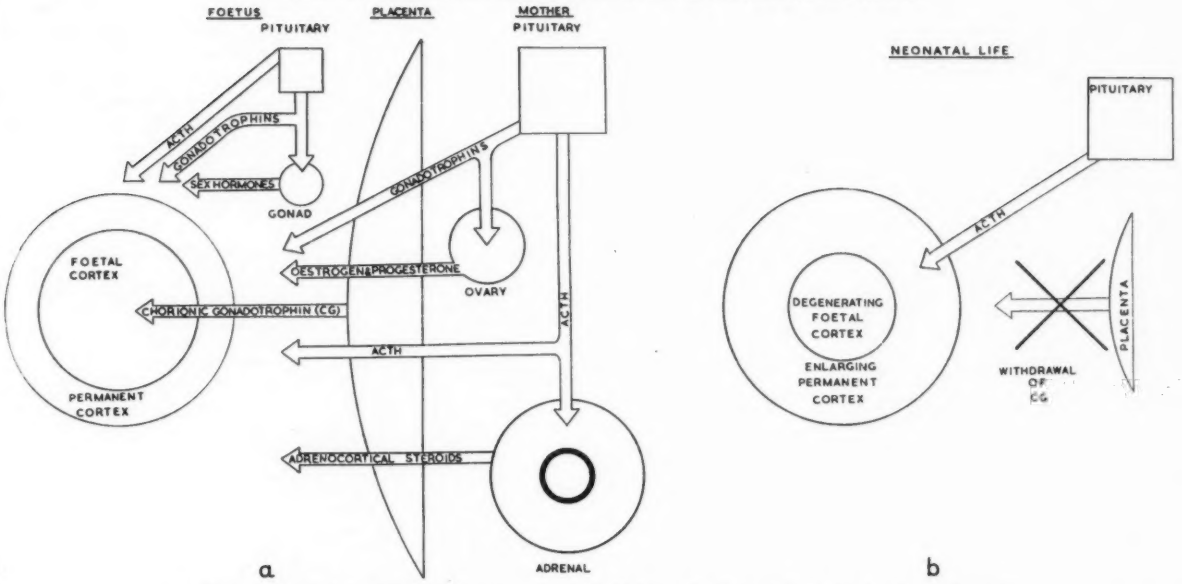
FIG. G. Anencephalous full-term baby. The adrenal comprises the permanent cortex with a small remnant of foetal cortex

(magnification × 85)

FIG. H. White female baby aged 51 days, with pseudohermaphroditism and evidence of massive androgen secretion; it died in an Addisonian crisis. The gland is hypertrophic, with an obvious outer and inner zona fasciculata, zona reticularis and a completely degenerated foetal cortex. A medullary connective tissue capsule has formed and the medulla is particularly well developed

(magnification × 85)

FIG. 2. ENDOCRINE RELATIONS OF THE ADRENAL CORTEX



a: showing some of the possible influences, none of which may be operative, on the developing human adrenal

b: illustrating the enlargement of the permanent adrenal cortex after birth, with the concomitant degeneration of the foetal cortex. It is suggested here that the withdrawal of chorionic gonadotrophin is the prime factor in this degeneration. There is, in fact, no evidence for hormonal influence on, or secretion by, the human foetal cortex

foetal endocrine secretion and its relationship with maternal hormones (fig. 2). The foetal cortex has been supposed to perform a variety of functions (Lanman, 1953; Baar, 1954), though none has been shown. Following on from the demonstration that the mouse adrenal X zone was responsive to pituitary LH, the hypothesis that the human foetal cortex was similarly influenced became more attractive (Gardner & Walton, 1954), as did the older theory that the zone was androgenic (Grollman, 1936). There is still no evidence for these suggestions, and certainly cells stained by the ponceau-fuchsin method (see Broster & Vines, 1938) are too widely distributed to be androgenic (Blackman, 1946). The claim of one hormone to be concerned with the foetal cortex has been overlooked. The foetal cortex seems to be confined to the Anthropoidea (the evidence for the Carnivora is weak; see Hill, 1937), and its uniqueness is matched by chorionic gonadotrophin (CG), which is LH in nature. Furthermore, CG is at its maximum early in pregnancy and declines later (Bruner, 1951). This correlates with the observed degeneration of the foetal cortex at a variable interval from full term (Keene & Hewer, 1928). The anencephalic monster has often been cited in support of the theory that the foetal pituitary controls the foetal cortex, on the grounds that the adrenal in these cases has a normal type of permanent cortex but that the foetal cortex is poorly expressed (Plate V, fig. G; and see Tähkä, 1951). Angevine (1938) considered that there was no correlation between the foetal pituitary and the size of the adrenal in anencephalics. It would seem, moreover, from Meyer (1912) that the anencephalic foetal cortex is normal until the fifth month, and the small neonatal adrenal may therefore be due to in-utero degeneration. If we suppose that CG influences the first conglomeration of coelomic epithelial cells that form the foetal cortex, then it would follow that the anencephalic has varying amounts of foetal cortex because of an early decline in CG secretion by the placenta. On this hypothesis, in anencephaly failure of the usual development of brain and adrenal is not cause and effect but arises from a common cause, namely placental abnormality.

5. The Adrenogenital Syndrome

It is doubtful if the adrenal cortex normally produces sex hormones. Certainly the castrate gives no biological evidence of androgen production, despite some 17-ketosteroid excretion. There is evidence that the adrenal can influence structures in the way expected of oestrogens (Mandl, 1951) and that the adrenal of young rats can at best secrete minute amounts of androgens transiently (Price, 1936; Hébert, Tran-Vy & Verne, 1954). There is never in the experimental animal, apart from adenomas (Woolley, 1950), that unequivocal and copious adrenal secretion of sex hormones so characteristic of the adrenogenital syndrome. This disease, first described by Bulloch & Sequeira (1905), occurs in many forms (Parkes, 1945). In the infant there is an early onset of virilism in the male and pseudohermaphroditism in the female. It would be attractive to regard the heavy adrenal androgen secretion as arising from a persistent and active foetal cortex. All the evidence points, however, to a rapid degeneration of the foetal cortex in macrogenitosomia praecox. It seems rather that the hypertrophy of the adrenal in these

cases depends to a large extent on the secretion of ACTH and perhaps of gonadotrophins (Plate V, fig. H). In some way the enzyme systems must be catalysed to secrete androgens rather than corticoids, the absence of which may result in manifestations of Addison's disease in infants showing the adrenogenital syndrome.

The adrenogenital syndrome demonstrates the extreme case of a general overlap between gonad and adrenal secretions, foreshadowed, but not inevitable, by the common embryological origin of the glands. Thus, in addition to gonadal secretion from the adrenal, there is the possibility of adrenocortical-type hormone production from the ovary and the heterologous action of the pituitary trophins involved. All this means that we must seek both chemical and biological definitions of hormones, and that caution must be used in interpreting experiments which, of necessity, underline one set of actions out of the context of the balanced endocrinological systems normally operating.

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HORMONES AND HUMAN FERTILITY

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1 Male fertility
2 Female fertility
3 Adrenal hyperplasia and fertility
4 The thyroid and fertility
5 Conclusions
References

The employment of medicinal substances for the promotion of human fertility is undoubtedly very ancient. Until comparatively recent times, when knowledge of the physiology of reproduction has permitted some approach to the diagnosis of the causes of infertility, all such treatment must, of course, have been empirical and undoubtedly was largely or wholly ineffective. With the growth of knowledge of the hormones and of their actions, the hope has often been expressed that suitable endocrine treatment might be of great value in the promotion of human fertility. It is not unfair to say that these hopes have, for the most part, remained unfulfilled; and that much of the hormonal treatment that has been employed has continued on an empirical basis, and has had little foundation in physiological fact. In this paper, no attempt will be made to deal with the past, and the survey of the use of hormones in human fertility will be confined to the present position.

It is proposed first to deal with the problems specific to male and to female infertility, and then to discuss one or two problems which are common to both sexes.

1. Male Fertility

For the promotion of male fertility, hormone preparations have been used for three purposes: to promote sex drive; to promote spermatogenesis; and to improve spermatozoal motility. Physiological matters only are dealt with in the following discussion. Other aspects are dealt with by Kinsey, Pomeroy & Martin (1948) and Kinsey, Pomeroy, Martin & Gebhard (1953).

Spermatogenesis. Much hormone therapy has been employed in the attempt to overcome defective spermatogenesis, mostly with little success. Theoretically, it is only when hormonal insufficiency is responsible for the failure of spermatogenesis that useful results can be expected from hormone treatment; in general, this is borne out in practice. Since in most subfertile men no hormonal deficiencies are apparent, while testicular biopsies frequently reveal patterns of tubular disorganization unlikely to be affected by any form of treatment, it is not surprising that hormone—and other—treatment seldom produces significant results. However, gratifying results of hormone therapy have been obtained in the treatment of hypogonadotrophic eunuchoids. Thus, Maddock, Epstein & Nelson (1952) reported on three such men who received 5,000 i.u. of chorionic gonadotrophin thrice weekly for three or more months: in one of these patients a testicular biopsy showed spermatogenesis proceeding to all stages but in another it proceeded only to the spermatocyte stage. (Curiously, in all three patients a rise in the initially low urinary oestrogens to levels several times above the normal was observed following treatment. The 17-ketosteroids also rose but did not exceed normal values.) Simmons (1952) treated four similar eunuchoids with chorionic gonadotrophin and sublingual methyl testosterone; both the wives of the two married men in this group became pregnant. More recently, Simmons (1954) has increased his series of these patients to 11, all of whom now have normal semen.

It seems possible that, in some instances, treatment of male eunuchoids with testosterone may also lead to spermatogenesis as well as to the development of normal secondary sex characters. Hurxthal, Bruns & Musulin (1949) described four patients who showed such a response; one of these subsequently had two children and another also had a child (the other two patients were unmarried). A similar response to testosterone, with the development of full spermatogenesis which persisted long after the last implantation, was reported by Swyer (1951). This was in a man who, at the age of 27, still had genital development only of a degree to be expected in a boy of about 14.

The objection has often been raised that the alleged results of treatment in these patients are, in fact, no more than the spontaneous development of late puberty, which occurred—or would have occurred—in spite of treatment. It is unlikely that this is true for all the patients reported. For example, one of the patients of Hurxthal and his colleagues was still eunuchoidal at the age of 43; he became fertile after testosterone treatment, and the possibility that this change was independent of the treatment seems far less likely than that it was caused thereby. Similar arguments apply to a number of the other reported cases.

It is when we come to consider the uses of hormones in dealing with defective spermatogenesis, or deficient spermatozoal activity, in non-eunuchoidal men, that disappointment and controversy appear. Although much use has been made of the available gonadotrophins—chorionic and equine—and considerably less of pituitary preparations which are not commercially available, it is fair to say that the results achieved have been almost universally negligible. Particularly is this so when due account is taken of the marked variability in semen samples shown by many men; unless a proper datum-line is secured by repeated examination before treatment, any apparent improvement after treatment has no significance. This objection can be raised to some of the favourable reports in the literature both on gonadotrophic and other forms of treatment of seminal deficiencies.

Testosterone has been employed for the treatment of defective spermatogenesis in two ways: administration in high dosage to produce temporary testicular suppression, to be followed by a rebound on cessation of treatment; and administration in low dosage for an expected direct " stimulatory " action on spermatogenesis.

The historical background to what is called the " rebound effect " of testosterone in high dosage is as follows: for many years it has been known that testosterone in sufficient dosage would depress spermatogenesis, and in 1950 Heller, Nelson, Hill, Henderson, Maddock, Jungck, Paulsen & Mortimore

showed by serial testicular biopsies that the initial degenerative changes immediately following the course of treatment were succeeded by repair and, in some patients, the development of a testicular structure appreciably better than before treatment. In a series of papers Heckel and his colleagues (Heckel, 1951; Heckel, Rosso & Kestel, 1951; Heckel & McDonald, 1952a, 1952b) reported the results of testosterone administration to 79 subfertile men, of whom 72 were " oligospermic " (though the total number of spermatozoa in ejaculates before treatment was as high as 60 million in some patients) and seven were azoospermic. In all 72 oligospermic men, testosterone caused depression of spermatogenesis. From the data on 45 of the men, it was concluded that 23 patients showed a rebound phase, the sperm count rising well above the levels before treatment, though it subsequently fell again in seven patients; no rebound phase was observed in 22 patients; there had been 10 pregnancies, of which five had resulted in healthy babies, and two had terminated in abortion at four months by the time of reporting.

These claims by Heckel and his colleagues naturally aroused considerable interest, and many workers have now used testosterone in the same way. Although there have been few further reports in the literature, it is well known that the general reaction has been one of disappointment. Simmons (1954) states that the replies to over eighty questionnaires sent to members of the American Society for the Study of Sterility were unfavourable to the rebound technique. In the writer's limited experience with this technique, a rebound has indeed been found in a few cases, but these have comprised less than 20% of the men so treated, and certainly not the 50% reported by Heckel & McDonald (1952a).

There is some evidence that testosterone administration in low dosage, by the implantation of pellets, may have a beneficial effect on spermatogenesis. Okbi (1949) implanted two pellets of 100 mg. each in ten men complaining of sterility and obtained what appeared to be improvement of the seminal fluid in eight of them. However, since repeated analyses before treatment were not made in most of these cases, the conclusions are open to doubt. A more extensive series was reported by Swyer (1953a, 1953b); in this, the effect of implanting two and three 100 mg. pellets of testosterone subcutaneously was studied in 56 men presenting seminal deficiencies of one kind or another. These men received a total of 67 implants—28 of 200 mg. and 39 of 300 mg. The seminal fluid appeared to improve in 28 individuals (12 received 200 mg., 16 received 300 mg.); to be unchanged in 22 men; and to deteriorate in 6 others (1 received 200 mg., 5 received 300 mg.). No significant effects on volume and seminal morphology were seen but there was a highly significant increase in motility with both doses of testosterone, and in density with 300 mg. implants. Extreme oligospermia was invariably unaffected by the treatment. The wives of 20 men became pregnant on 22 occasions, all within 6 months of implantation; 5 pregnancies ended in abortion (22.75%), the remainder going to term. It was suggested that moderate depression of sperm density (15–20 million/ml.) or poor motility (other than extreme asthenospermia) with fair or good density offered the best chances of improvement after testosterone implantation.

Spermatozoal motility. Extremely little is known about the factors necessary for the development of normal spermatozoal motility, and for the persistence of activity. Consequently, the causes of deficient sperm activity are unknown, treatment is empirical, and the results correspondingly disappointing. The writer knows of no means of influencing persistent true necrospermia (wherein motile spermatozoa are never found). Douglas (1946) found that testosterone was often helpful when the sperm count was normal but activity was deficient. More convincing evidence of this was obtained by Swyer (1953a, 1953b; see above). Sometimes the administration of desiccated thyroid helps; the use of thyroid will receive further comment later.

2. Female Fertility

In addition to the purely empirical and non-specific use of hormones for the promotion of fertility in women, about which nothing further will be said, various endocrine preparations have been employed for the following main purposes in the treatment of female infertility: the correction of genital hypoplasia; menstrual disturbances, leading to the failure of ovulation or its infrequent occurrence; endometrial dysfunction, causing nidatory failure; threatened and repeated abortion; and for the prophylaxis of late pregnancy toxaemia.

Genital hypoplasia. This condition is probably diagnosed much too frequently and on inadequate evidence. Moreover, when it really does exist, unless accompanied by other evidence of ovarian insufficiency, it is most commonly due to target organ failure and is therefore scarcely amenable to treatment. However, the administration of oestrogens in moderately high dosage and in interrupted courses does sometimes lead to growth of the uterus and cervix, which may increase the chances of conception at a later date.

Menstrual disturbances. A full treatment of this subject is clearly out of the question. The use of oral hormonal therapy for various menstrual irregularities has been described by Swyer (1950). The literature is replete with reports favourable and unfavourable on the use of various hormone treatments for the promotion of ovulation in women. A valuable review from the physiological aspect is that of Hisaw (1947). From among the voluminous literature on the effects of gonadotrophin treatment the following may be mentioned: Davis & Koff (1938), Hartman (1938), Geist, Gaines & Salmon (1941), Greenblatt (1941), Mayer & Ravetz (1941), Davis & Hellbaum (1944), Abarbanel & Leathem (1945), Davis & Hamblen (1945), Hamblen & Davis (1945), Morton (1946), Kurland (1951) and Bergman & Wahlén (1952). In a recent survey of the use of steroids and gonadotrophins in gynaecology, Taylor (1953) has divided the indications for these various substances into " acceptable ", " probable occasional ", and " doubtful " categories; so far as gonadotrophins are concerned, all the indications he regards as doubtful, listing sterility due to failure of ovulation as number one. Though most critical studies have failed to demonstrate conclusively the ability of gonadotrophins to stimulate ovulation in women, it is admitted that the occasional occurrence of pregnancies during or following a course of gonadotrophin treatment keeps open the possibility that under special circumstances the desired result may actually be produced.

Disturbances of cervical function. Disordered cervical function, by preventing the ready ingress of spermatozoa into the cervical canal and their survival therein, is a common contributory cause of infertility. Failure, at the time of ovulation, to develop a free flow of watery mucus into which the spermatozoa may penetrate, and within which they may survive for prolonged periods of time, may be the result of a relative

oestrogen deficiency, or, perhaps more likely, of a relatively inadequate endocervical response to otherwise normal oestrogenic stimulation. It is common practice to administer small doses of oestrogens during the first half of the menstrual cycle, when such a condition is found, and there can be no doubt that this treatment often improves the flow of cervical mucus at the time of ovulation, previously negative post-coital tests becoming positive. A practical difficulty of this treatment may be that the dose of oestrogen required to improve the cervical mucus is sufficiently large either to inhibit or to postpone ovulation. When this is the case, the more usual course of oestrogen, beginning about the fourth day of the cycle and continuing for 10–14 days, can be replaced by a much shorter course beginning three or four days before the expected day of ovulation, but consisting of a higher dose, such as 2 mg. of stilboestrol daily, instead of 0.2–0.5 mg. daily as given over the longer course.

Endometrial dysfunction : nidatory failure. Routine endometrial biopsies, taken shortly before the menstrual period is expected, occasionally reveal a degree of development corresponding to a phase of the cycle earlier than the immediately premenstrual phase. These findings are usually interpreted as being indicative of progesterone deficiency, or of deficient endometrial response to normal progesterone levels, and it is generally agreed that in such circumstances failure of nidation of a fertilized ovum may be the cause of sterility. It is doubtful if this is a common or important infertility factor, and it is quite uncertain that pregnancies in such patients, following the administration of progesterone or ethisterone during the second half of the cycle, in order to increase the degree of secretory development, are, in fact, to be attributed directly to the treatment. It is a matter of experience that a biopsy specimen indicative of progesterone deficiency may later be followed by an entirely normal one, without any treatment having intervened. However, when repeated premenstrual biopsies show a real deficiency of progestational development, it is quite reasonable to administer progesterone in some form or other during the second half of the cycle, and when this is done, the biopsy may assume a more normal pattern. In these circumstances, the administration of the hormone would be a reasonable measure for the promotion of fertility.

Threatened and repeated abortion. It has long been a popular belief that spontaneous abortion is usually due to hormonal deficiencies and, as a corollary, the exhibition of various hormones, mostly progesterone, oestrogens or desiccated thyroid, has been extensively advised in an attempt to deal with these conditions. Opinions vary greatly on the efficacy of the hormone treatment of spontaneous abortion. This is not surprising, since it is probable that hormonal deficiency is a relatively uncommon cause of abortion. The remarkable studies of Hertig & Rock (1949) suggest that germ plasm defects of various kinds are probably a far more common cause of spontaneous abortion than are endocrine factors. On the other hand, Peters, Man & Heinemann (1948) found evidence of a decrease in the serum protein-bound iodine (PBI, a measure of circulating thyroid hormone) in some women threatening to abort; and in seven patients, in whom PBI concentrations of less than 6 μg./100 ml. were discovered early in pregnancy, thyroid treatment raised the level to normal in all cases, the pregnancies continuing without interruption—as, of course, they might have done anyway. Moreover, Bender (1948) has shown that when progesterone is given to women with low pregnanediol excretions who are threatening to abort, the salvage rate is materially increased; while when it is withheld from women with high pregnanediol levels who are threatening to abort, a similar improvement in salvage rate is obtained. Some of the French workers, notably Jayle & Crépy (1954), believe that hormonal insufficiency, be it of oestrogens or of progesterone metabolites, or both, may commonly occur in pregnancies threatening to abort; these authors believe that hormone treatment should be based on the results of urinary steroid analyses—oestrogen, progesterone, or both, being given according to the apparent needs.

Both oestrogens and progesterone have been used for the prophylactic treatment of repeated or so-called habitual abortion. Smith (1948), using stilboestrol according to an incremental dosage scheme, and Bishop & Richards (1952), using progesterone implants, obtained a success rate of the order of 80%, but so did Bevis (1951) who used no specific treatment, but merely subjected his patients to extensive investigation. Swyer & Daley (1953) could find no significant difference between the results in a control group and those in a group treated with progesterone implants, using the same technique as Bishop & Richards (1952). Nor could they find any convincing evidence of progesterone deficiency in these patients. On the other hand, Jones & Delfs (1951), after studying extensively the endocrine patterns in women with histories of abortion, based their treatment (with thyroid, progesterone and vitamin E) on the findings of apparent deficiencies of these factors; 85% of the treated hormone-deficient patients went to term, as compared with only 22% of the untreated hormone-deficient patients. But, since only 50% of the untreated patients not deficient in hormone went to term, it seems quite possible that the treatment may have had a psychological, as well as—or even instead of—an endocrinological effect (see also Speert, 1954).

Prophylactic antenatal use of stilboestrol. Smith & Smith (1949) claimed that the antenatal administration of stilboestrol according to an incremental dosage scheme to 387 primigravidae had several significant beneficial effects on the course and outcome of pregnancy as compared with those of 555 untreated " controls ". The incidence of toxaemia in late pregnancy was reduced, and when toxaemia occurred, it did so later in the pregnancy and was less severe. Prematurity and postmaturity were less frequently encountered, and the incidence of unexplained stillbirths and foetal deaths was reduced. Until recently, this work had been neither confirmed nor denied, but Ferguson (1953), on repeating the experiment with more adequate controls, could find no effect on pre-eclampsia, prematurity and foetal survival in his 199 treated patients as compared with 203 control patients given placebos. Still more recently, Dieckmann, Davis, Rynkiewicz & Pottinger (1953) have reported comparable results in a large and well-controlled series, while Swyer & Law (1954) have also been unable to find any difference between control patients on placebos and patients treated with stilboestrol.

3. Adrenal Hyperplasia and Fertility

Following the remarkable studies of Wilkins and his colleagues (Wilkins, Lewis, Klein & Rosemberg, 1950a, 1950b; Wilkins, Lewis, Klein, Gardner, Crigler, Rosemberg & Migeon, 1951; Wilkins, Crigler, Silverman, Gardner & Migeon, 1952a, 1952b; Wilkins, Gardner, Crigler, Silverman

& Migeon, 1952) on the effects of cortisone administration to patients with congenital adrenal hyperplasia, a new interest has arisen in the possibility that certain forms of sterility, both male and female, may be due to this cause and may therefore be amenable to hormone therapy. Jones, Howard & Langford (1953) suggested that women with symptoms indicative of mild adrenal dysfunction, such as amenorrhoea or oligomenorrhoea, hirsutism, acne and slightly elevated 17-ketosteroid excretions, might respond to cortisone therapy by ovulation. Jones & Jones (1954) have recently elaborated this subject further, distinguishing four groups of cases with congenital adrenal hyperplasia: (i) female pseudohermaphroditism, (ii) post-natal virilism, due to adrenal hyperplasia, (iii) post-puberal hirsutism, infertility and oligomenorrhoea with elevated 17-ketosteroid excretion, and (iv) as in (iii), but with normal 17-ketosteroid excretion. Examination of the enlarged adrenals from 15 patients in groups (i) and (ii) showed hyperplasia of the reticular zones, with lack of the zona fasciculata. In normal adrenals, the latter zone contains birefringent lipids and the former does not; but, in these hyperplastic adrenals, the distribution is reversed. It is the fascicular zone that produces the glucocorticoid type of hormone, so that absence or functional incompetence of this zone is the cause of the disturbed hormonal pattern. Failure adequately to convert steroid precursors into glucocorticoids leaves the pituitary uninhibited; in consequence, adrenocorticotrophic hormone is secreted in excess, causing the adrenal over-activity with the production of aberrant androgenic steroids, of which pregnanetriol may be the most important. The condition can be corrected by the administration of cortisone. With a daily dose of 50 mg. orally for the first month, followed by 25 mg. daily subsequently, ovulation was induced in all 16 patients in group (iii), and 5 out of 10 conceived. Of the patients in group (iv), all have ovulated and, to date, all but 4 out of 11 had conceived.

The studies of Wilkins & Cara (1954), on the effects of cortisone therapy on testicular development in boys with virilism due to adrenal hyperplasia, encourage one to hope that this hormonal treatment will prevent the sterility which is otherwise found in adults who have suffered from this condition.

4. The Thyroid and Fertility

Writing in 1936, Chute stated:

> . . . Last, but far from least, the oral administration of thyroid is indicated in all cases presenting even a low normal metabolism, and obesity. In some way it also seems to act as a most valuable adjuvant in pituitary-gonad cases, possibly because it activates sluggish bodily processes. It is one of the most valuable aids in sterility, either alone or in combination with other therapy . . .

Kreutzmann (1940), discussing sterility in the male, was also impressed with the value of thyroid medication. He found 15% of 45 men to have a basal metabolic rate between −10% and −18%; thyroid therapy was claimed to have produced pregnancies by all of these men! Many other subsequent writers also appear to have been favourably impressed by the value of thyroid medication, mostly on purely empirical grounds. Critical studies, however, are almost totally lacking in the literature, and a salutary paper by Tyler (1953) entitled "The thyroid myth in infertility" puts the case very strongly against the indiscriminate administration of desiccated thyroid or thyroxine in cases of infertility, and the facile supposition that subsequent pregnancies are the direct result of this treatment. It is doubtful if significant hypothyroidism is any more prevalent among persons complaining of infertility than among the rest of the population (Peters and his colleagues, in 1948, could find no evidence for it in terms of lowered serum PBI), and it is certain that indiscriminate administration of thyroid hormone, either in large or in physiologically insignificant doses, is wholly to be deplored.

5. Conclusions

Clearly defined endocrine causes of infertility are relatively uncommon. When found, certain of these are amenable, to a greater or less extent, to hormonal treatment. In the absence of endocrine infertility factors, the empirical administration of hormones has little or no effect on the promotion of fertility, and can only be condemned.

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HORMONES IN RELATION TO FERTILITY IN FARM ANIMALS

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1 Hormone balance
2 Increasing the ovulation rate
3 Breeding during the non-breeding season
4 Speeding up the age of puberty
5 Effects of nutrition on hormone action
6 Transplantation of fertilized ova
7 Hormones and uterine infection
8 Effects on the male
References

In this survey references are given only to selected papers which may be consulted for further information.

The number of eggs shed at each ovulation depends on the level of gonadotrophic hormones from the anterior pituitary gland circulating in the blood. If one ovary is removed from rabbits which normally shed five eggs from each ovary, the remaining ovary will shed ten eggs (Asdell, 1924). Similarly, if in such rabbits gonadotrophic hormones from the anterior pituitary are injected subcutaneously, 20 to 50 eggs or more, according to the dose given, will be shed (Pincus, 1940).

The two main gonadotrophic hormones concerned are (i) follicle-stimulating hormone (FSH) which, given subcutaneously or intramuscularly so as to have a slow continuous action, causes the follicles to ripen, and (ii) luteinizing hormone (LH) which, given intravenously so as to have a quick action, causes the follicles to rupture, liberate the eggs and form corpora lutea.

1. Hormone Balance

Comparison of the different species of farm animals shows that the balance between these two hormones in the anterior pituitary gland has a profound influence on the reproductive processes in the animal, as shown in Table I (Hammond, 1953).

TABLE I. EFFECTS OF THE RELATIVE AMOUNTS OF FOLLICLE-STIMULATING HORMONE (FSH) AND LUTEINIZING HORMONE (LH) IN THE ANTERIOR PITUITARY GLAND ON THE OESTROUS CYCLE

Anterior pituitary	FSH (oestrogens) ⟶ LH (progesterone)			
Species . .	Mare	Sow	Ewe	Cow
Duration of oestrus (hr.) . .	144	72	24	16
Ovulation (hr. in relation to end of oestrus) . .	38 (before)	4 (before)	0 (after)	14 (after)
"Silent heats"*	Rare		Frequent	

* The occurrence of ovulation at the normal time without the symptoms of oestrus.

Thus, at one end of the scale, the mare with much FSH and little LH in the anterior pituitary has a prolonged oestrous period, caused perhaps by the low level of LH, and ovulates some 24–48 hours before the end of oestrus, whereas at the other end of the scale the cow with much LH and little FSH in the anterior pituitary has a short oestrous period and ovulates some 14 hours after the end of oestrus. The other two species are intermediate, the sow being more like the mare and the ewe more like the cow.

Under conditions of lowered anterior pituitary activity the hormone in shortest supply is usually the cause of the sterility involved. Thus, in a cold dry spring, the mare with its low level of LH has difficulty in ovulating and oestrus may persist for 30 days or more, and so the sperm die, before ovulation occurs. Under such conditions intravenous injection of LH at the time of mating causes ovulation within 30 hours and so increases fertility (Day, 1939a; Davison, 1947). On the other hand, during winter the young cow—with its high level of LH in proportion to FSH—ovulates very readily, so that the duration of oestrus is very short, or ovulation may even occur before oestrus has had time to appear and so cause what is

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known as a " silent heat ". These " silent heats ", at which ovulation occurs but at which no signs of oestrus are observed, can be overcome by squeezing out the corpus luteum from the ovary per rectum about the seventh to tenth day of the cycle and so, by ripening a follicle soon afterwards, reducing the amount of LH stored (Marshall & Hammond, 1952).

While much sterility in the mare is due to follicular cysts (Day, 1939b), much sterility in the cow is due to persistent corpora lutea or luteinized follicular cysts causing failure of oestrus to appear (Hammond, 1927), this difference between species being caused by differences in the hormone balances of their anterior pituitaries. In this respect again the sow is more like the mare, as among the main causes of infertility in the sow are follicular cysts (Pomeroy, 1952), while the ewe is more like the cow in that there is some evidence that luteinized follicles are not uncommon (Robinson, 1950, 1951; Averill, personal communication, 1954). Most of the so-called follicular cysts in the cow are really luteinized follicles in that they inhibit the occurrence of oestrus.

2. Increasing the Ovulation Rate

The FSH hormones have been used to increase the number of eggs shed in cows and ewes (Zavadovsky, 1941) in an attempt to increase the number of young produced at a birth. Since, if many more than the normal maximum number of eggs are produced, the surplus embryos die and are absorbed, mainly before attachment (Robinson, 1951), effort has been mainly directed to find a dose and method which would give the normal maximum number of eggs shed. In most of these experiments the source of the FSH has been from pregnant mare serum (PMS) rather than from horse anterior pituitary, not only on account of the cost but also because it is not so easily destroyed in the body. One injection of PMS will act for four or five days whereas FSH derived from the anterior pituitary is more easily destroyed and so requires priming doses given every 12 hours to be effective.

In cows it has been found (Hammond, jr & Bhattacharya, 1944; Hammond, jr, 1949) that some 2,500 i.u. of PMS will on the average give twins, with some singles and triplets, if injected either about four days before oestrus is due or about two days before the removal of the active corpus luteum per rectum. Since by these methods the detection of a previous oestrous period or two visits to the farm is necessary, Rowson & Lamming (personal communication, 1954) have tried with success another method, that of injecting the PMS immediately after the active corpus luteum has been squeezed out per rectum. If FSH is injected while an active corpus luteum is present in the ovary, ovulation is prevented and large follicular cysts are formed (Folley & Malpress, 1944).

With sheep, Robinson (1950) found that a small dose of about 450 i.u. of PMS, injected about four days before oestrus was due, raised the fertility of the treated ewes to 167 per 100 ewes mated as compared with 147 for the controls. Wallace (1954) and Gordon (personal communication, 1954) have confirmed these results, but find that the exact dose required varies with the breed and other conditions. Both Robinson (1951), working with sheep, and Dowling (1949), working with cattle, found that a large dose may cause the shedding of very large numbers of eggs which frequently pass down the tubes so quickly that fertilization does not occur. Rowson (1951) found that, in the presence of a corpus luteum or after injection of progesterone (when LH is used to cause ovulation), the ova travel down the tube at a greatly increased rate.

The number of follicles produced in cows as a result of a given dose of PMS, assayed by the rat method, is variable, and attempts have been made to determine the cause. Rowson (1951) found that, following the injection of a similar dose, more follicles (average 26) were ripened from raw PMS stored at -10° C. than from dried purified preparations (average 14). It may well be that the raw serum is not so easily destroyed by the body and therefore has a more prolonged action.

The percentage of FSH-induced follicles which will ovulate spontaneously in the cow is related to the time elapsing from injection to the occurrence of oestrus; for example, Brock & Rowson (1952) found that, when this was two days the percentage of follicles which ovulated was 11.6, after four days 23.1, and after five to 11 days 82.6. They found, however, that if the follicles were stimulated for too long (more than five days) before ovulation some of the eggs when shed appeared to be dead and so incapable of fertilization; Dowling (1949) had previously described such eggs after PMS injections. If an active corpus luteum is present in the ovary, the follicles induced by PMS injections fail to ovulate unless LH is injected intravenously. The percentage of the follicles which then ovulate increases with the interval between the PMS and LH injections, from 61 with an interval of four days to 89 with an interval of seven days (Brock & Rowson, 1952). Unfortunately this high ovulation rate in this case is of little practical importance as it has been found impossible to fertilize the ova shed when there is an active corpus luteum in the ovary.

3. Breeding during the Non-Breeding Season

Some species of farm animals have a non-breeding season during which follicles are not normally ripened. As shown by another review in this series[1], this anoestrus is related to the ratio of hours of darkness to hours of daylight which affects the anterior pituitary gland, some species (mare, ferret) responding to an increased light ratio and others (sheep, goat) responding to an increased dark ratio. Since the control of daylight hours is a rather expensive method of prolonging the breeding-season in sheep, attempts have been made to do this by hormone injections. It has been shown (Hammond, jr, Hammond & Parkes, 1942; Hammond, jr, 1945) that injections of FSH made during the non-breeding season in sheep seldom fail to cause ovulation, but only in a few cases cause a coincident oestrus and so a possibility of conception. In these few cases it was found that a fairly recent corpus luteum existed in the ovary, for occasional spontaneous ovulation without oestrus sometimes occurs in the ewe during the non-breeding season. By giving a second injection of FSH some 12 to 14 days after the first, it was found that an increasing proportion of ewes come into oestrus and, if served, conceive (Cole & Miller, 1933; Hammond, jr, *et al.* 1942; Hammond, jr, 1945; Robinson, 1950), but the proportion was not sufficient to justify commercial application. Robinson (1950) found that the proportion of ewes which came into oestrus and ovulated after one injection could be increased by injection of testosterone before the injection of PMS, and later (Robinson, 1954) that a high proportion of ewes ovulated after injection of 6.25 mg. of

[1] See Amoroso & Matthews, p. 87 of this number of the Bulletin.—Ed.

progesterone twice daily for six-and-a-half days before the injection of 1,000 i.u. of PMS, came into oestrus and conceived during the non-breeding season. Gordon (personal communication, 1954) has confirmed these results and shown that even ewes during the lactation anoestrus can be made to conceive by this method. In this way ewes can be made to produce two crops of lambs a year.

4. Speeding up the Age of Puberty

Another application of gonadotrophic hormones in animal production is that of speeding up the age of puberty, so that reproductive life can begin earlier and genetic improvement can be accelerated by shortening the time between generations. It has been found that the earliest age at which injected FSH will ripen follicles in the ovaries is one week for calves (Marden, 1953), one month for lambs (Mansour, personal communication, 1954) and three months for rabbits (Adams, 1954). This sequence corresponds with the degree of physiological and anatomical maturity of the species at birth and depends on the age at which follicles with antra first appear in the ovaries; this, as indicated below, depends on the nutritive demands of other tissues of the body. At this early age it is found too that the follicles induced by FSH injections do not ovulate at all easily. Marden (1953) found that, although ovulation can occur spontaneously in the FSH-treated calf before the end of the first month of postnatal life, only 65% of the calves ovulated, although many follicles were produced. In order to obtain multiple ovulation he had to repeat the FSH treatment a second time towards the end of a cycle of luteal activity, when, instead of one or two follicles ovulating, almost all the follicles of any size in the ovaries ovulated, reminding one of the luteinization of all the follicles that takes place in the mare's ovary during the fourth month of pregnancy. Rowson (1951) too found that in mature cows a larger proportion of the follicles ruptured in the presence of a waning corpus luteum than in its absence.

The ova produced from such FSH-induced ovulations in the very young animal are capable of fertilization but, owing to the infantile conditions of the reproductive tract, they fail to develop (Marden, 1953, for calves). However, if such fertilized ova are transplanted to adult rabbits in the appropriate stage of the cycle they develop normally (Adams, 1954). When these young does become a little older, a few small, weak young may be produced whereas, if some of their eggs are transplanted to adult does, large, strong young are produced. These experiments indicate that the onset of puberty is a gradual process: the various hormones concerned with the ripening of follicles and with ovulation, and those necessary for the maintenance of pregnancy, come into action in an orderly sequence.

5. Effects of Nutrition on Hormone Action

Evidence is accumulating that the nutritional status of the animal has an effect on the response to a given dose of gonadotrophic hormone. Adams (1953) found that rabbits reared on a high plane of nutrition would respond to injections of FSH at an earlier age than those reared on a low plane of nutrition, but if the latter were well fed for a week or two they would respond at a lower body-weight than those reared on a high plane. Marden (1953) too found that the ovary of poorly nourished calves would not respond to FSH injections even though massive doses were given, because of the absence of follicles with an antrum. It has long been known that by flushing ewes, i.e., by putting them on to a high plane of nutrition before mating, the percentage of lambs born can be increased (Nichols, 1926; White & Roberts, 1927). Whether the explanation for this is that the plane of nutrition increases the number of follicles with an antrum or that it increases the output of FSH from the anterior pituitary is still an open question (Wallace, 1954).

The implantation of tablets of the synthetic oestrogen stilboestrol, which has been done either for purposes of improving the quality of meat in lambs or causing lactation in virgin heifers, causes the ovary to become atrophic (Averill, personal communication, 1954, for lambs; Hammond, jr & Day, 1944, for heifers), probably by inhibiting the production of FSH by the anterior pituitary. In such cases, however, the injection of FSH fails to cause the ripening of follicles. This may be because under such conditions the whole nutritional level of the ovary is reduced so that no follicles with an antrum exist. In heifers, after the stilboestrol tablets are removed the ovary gradually begins to function again, but frequently, in this recovery stage, when the body is still under some oestrogenic influence, the cow behaves like the mare (see Table I), and ovarian cysts are formed (Hammond, jr & Day, 1944); as the influence of the oestrogen wears off, however, these are spontaneously resolved, and ovulation and normal cycles are resumed. In contrast, in the sow, in which the natural hormone balance is different from that of the cow (see Table I), ovarian cysts can be caused by keeping the animal under the influence of progesterone (Pomeroy, personal communication, 1954), this apparently still further depressing the already low level of LH in the anterior pituitary of this species.

6. Transplantation of Fertilized Ova

Attempts are now being made to exploit the effects of the gonadotrophic hormones in increasing the number of eggs shed at oestrus, by transplanting the surplus fertilized ova into other individuals. This has now been done for rabbits (Dowling, 1949; Adams, 1953), and the fertilized egg in the four-cell stage has been flown from the USA (in a thermos to control temperature) and transplanted successfully to does in England (Chang & Marden, 1954). Transplantation has also been successfully performed in sheep (Warwick & Berry, 1949), pigs (Pomeroy, Adams & Rowson, personal communication, 1954) and cattle (Willett, Black, Casida, Stone & Buckner, 1951). Hunter, Adams & Rowson (1955) have used this method to determine the maternal influence on the offspring by transplanting fertilized ova from large to small breeds of sheep and vice versa. In order to synchronize oestrus in donor and host, injections of progesterone are given to delay ovulation for the desired time (Hunter, 1954).

7. Hormones and Uterine Infection

During the course of experiments on egg transplantation in the cow, Lamming & Rowson (1952) found that when the eggs were inserted through the cervix by the technique used in artificial insemination—but at four days after oestrus instead of at oestrus—the uterus invariably became infected. In sets of ovariectomized cows injected with progesterone and oestrogen respectively and then inseminated with semen or bacterial

cultures through the cervix, Rowson, Lamming & Fry (1953a) demonstrated that the uterus could not be infected when under the influence of oestrogen, but could easily be infected when under the influence of progesterone. It seems possible that the explanation is that, when the tract is under the influence of oestrogen, the production of mucin forms a barrier to infection whereas, when it is under the influence of progesterone, the uterine milk produced to nourish the embryo produces an excellent culture medium for bacteria. This idea is supported by the observations of Laing & Day (1947) on cows infected with *Trichomonas foetus*, in which the disease was resolved by the occurrence of one or two oestrous periods, but exacerbated by the persistence of a corpus luteum. While by this means spontaneous recovery occurs in the cow, no case has yet been reported in which an infected bull has been cleared of the disease. Rowson, Lamming & Fry (1953b) have suggested that this is owing to the fact that testosterone has an action similar to that of progesterone. Since they found that they could override the action of progesterone in the cow by giving massive doses of oestrogen, they suggested that such treatment might cure bulls infected with *Trichomonas foetus* and *Vibrio foetus*. Preliminary experiments have shown promising results (Rowson, personal communication, 1954).

8. Effects on the Male

The reaction of the male to gonadotrophic hormones is very different from that of the female. In the female, when one ovary is removed the other ovary continues to produce the full number of ripe follicles characteristic of the species (Asdell, 1924), owing to the fact that the number which ripen depend on the level of FSH in the blood. In the male, on the other hand, removal of one testis halves the number of sperms produced in the semen (Edwards, 1940). The formation of ova in the ovary or of sperm in the testis is not influenced by FSH. Just as FSH acts on the follicle cells, causing growth of the follicle and production of oestrogen, so it acts on the supporting cells of the testis and increases its output of hormones. So far it has not been found possible to increase the sperm output of a bull by injecting PMS, but large doses (5,000 i.u.) of PMS will increase for a few days the sex-drive of many young bulls which hitherto have shown little desire to mate (Day, 1942). In species such as the boar, in which large quantities of vesicular secretion are produced, the implantation of stilboestrol tablets in the young animal will prevent the growth of the seminal vesicles and so reduce the volume of semen and increase the concentration of sperm in it (Wallace, 1949). Presumably this is owing to the stilboestrol reducing the output of FSH by the anterior pituitary (see also above for the cow) and consequently lowering the output of the testicular hormones which are responsible for growth of the seminal vesicles. For old boars in which the seminal vesicles are already formed the treatment is not so effective.

* * *

The effect of the various hormones on the maintenance of pregnancy will not be surveyed here as it forms the subject of another paper[2]; likewise the question of sperm transport is dealt with in section 3 of the paper by Cross[3] in this number of the Bulletin.

[1] See Amoroso, p. 117 of this number of the Bulletin.—Ed.
[2] See Cross, p. 151 of this number of the Bulletin.—Ed.

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Book Reviews

The Technique and Significance of Oestrogen Determinations

Edited by *P. Eckstein & S. Zuckerman*. Cambridge: University Press, 1955. (Memoirs of the Society for Endocrinology, No. 3.) 96 pages; figures. 25 × 19 cm. 18s.

This monograph is a record of the proceedings of a conference of the Society for Endocrinology held jointly with the Endocrinological Section of the Royal Society of Medicine. There were two sessions, each with four papers which are published in full together with the subsequent discussion of each paper individually. These are followed by a report on the general discussion which terminated each session.

The first two papers were by J. B. Brown and by W. S. Bauld, who were working in close collaboration in Edinburgh on the assay of oestrogens. Each had evolved his own technique while adopting some of the procedures devised by the other, but both started with acid hydrolysis of the urine and ended with a new development of the Kober reaction. They differed in methods of extraction and chromatography. Brown also described the clinical application of his method to the daily output of oestrone, oestradiol and oestriol, during the normal menstrual cycle and in early pregnancy in a number of individuals, and also after injection of oestradiol. There was a consistent time-lag of about 24 hours in the excretion of oestriol compared with oestrone and oestradiol. The next paper was by R. J. Boscott, who described the separation by paper chromatography of a great number of oestrogenic and other phenolic compounds in urine, and the subsequent identification of some of them. The influence of diet on these excretion products was discussed. The fourth paper was by H. Braunsberg, M. I. Stern & G. I. M. Swyer, who described a method for the urinary assay of oestrogens, which employed a fluorimetric method of estimation after acid hydrolysis and chromatography. The lively general discussion which followed was centred mainly upon the value of initial acid hydrolysis versus enzymic hydrolysis of urine, and on the final estimations employing the Kober reaction versus the fluorimetric method.

In the second session E. Diczfalusy described the application to human semen of his technique for oestrogen assay by use of solvent partition, counter-current distribution and fluorimetric analysis. A litre of human semen was found to contain approximately 60 μg. of oestrone, 10 μg. of oestradiol and 30 μg. of oestriol in the free form. Methods employed for the identification of these substances were described. Subsequent acid hydrolysis of the semen yielded no evidence for the presence of conjugated oestrogens, but alkaline hydrolysis gave small yields of "protein-bound" oestrone and oestriol. In the next paper F. L. Mitchell described the application of a method of paper chromatography for oestrogens, devised by I. E. Bush, to an extract of minced placental tissue. The sensitivity of the method, which was said to estimate 1 μg. of oestrogen in up to 200 g. of placental tissue, was compared with earlier methods which required 8 kg. of tissue in order to identify the oestrogens. Recovery experiments showed, unfortunately, losses of from 73% to 86%. It was suggested that the full-term placentae used in this experiment contained a factor that at some stage in the extraction or purification destroyed the oestrogens, but that the factor did not appear, from earlier work by Diczfalusy, to be present in early placentae.

The only paper on biological assay in the symposium was by P. M. Harlow, M. Sandiford & M. E. Wood. They used the response observed in the vaginal smear of ovariectomized mice treated with ether extracts of human urine after acid hydrolysis. The response was regarded as a measure of the combined action of oestrone and oestradiol. No estimate of the precision of the assay was given. The authors then described the application of this method to urine obtained during the normal menstrual cycle and in a number of pathological conditions.

The last paper was by P. M. F. Bishop, on the clinical application of oestrogen assays which, he considered, would be likely to remain research tools for a long time. The main interest in the final discussion was concerned with the significance of the oestrogen levels found in different fluids and tissues.

A. C. Crooke

Studies on Fertility: Including Papers Read at the Conference of the Society for the Study of Fertility, London, 1954. (Being Volume VI of the Proceedings of the Society)

Edited by *R. G. Harrison*. Oxford: Blackwell Scientific Publications, 1954. ix + 151 pages; plates; figures. 22 × 14 cm. £1 1s.

These are actually the Proceedings of the Society for the Study of Fertility, previous volumes of which were published only as a journal. Now they are deemed worthy of being issued in the form of a book. And if the professional standing of the contributors to the Proceedings of this Society be taken into consideration, this promotion to book form is well merited, for the Society numbers amongst its members almost everybody in Great Britain interested in the subject of sterility, whether from the human or the veterinary standpoint.

The subjects dealt with in this volume are so numerous and so varied that it would be difficult to make any general comment on them. Some of the papers, such as that on the histology of the testis of a rat exposed to exceedingly low temperatures, are of only scientific interest, whilst others are of importance to the clinician also. Amongst the more practical articles is one on the aetiology of male subfertility, a subject about which we have a great deal more to learn. It is no exaggeration indeed to say that no explanation can be offered in about half of the cases of subfertility seen in a fertility clinic.

This is the first volume of what we hope will prove to be a long and profitable series of books, and it has been produced under the able editorship of Dr R. G. Harrison, Professor of Anatomy in the University of Liverpool. The reviewer has only one criticism to offer the editor, and that an entirely constructive one. It is to the effect that it would be helpful to the general inexpert reader if the editor were to act as his friend and guide and provide him with a very brief indication of the chief advances made during the current year in the study of fertility. He would then be in a better position to appreciate the relative importance of the various articles.

Kenneth Walker

Reproduction and Sex

G. I. M. Swyer. London: Routledge & Kegan Paul Ltd., 1954. viii + 280 pages; illustrations. 22 × 14 cm. £1 5s.

When Leff's *Social medicine* (1953), the first volume of the Series of Human Biology, appeared, it became evident that a very intelligent and successful attempt was being made to satisfy a number of urgent needs. The present time is remarkable for the swift and vast expansion of scientific knowledge and for the dependence of our form of society for its continuance and further development upon the speedy and intelligent application of this knowledge in endeavours to promote human welfare. Such application in a society like our own is possible only when there exists among the population a widespread demand for it, this demand arising from an understanding of what is involved and of what the outcome of such application is likely to be. This series is concerned with these very matters. It is addressed to (i) such as take a serious interest in human affairs and wish to keep themselves accurately informed on relevant topics; (ii) specialists in different branches of knowledge who wish to keep abreast of advances in fields related to their own; and (iii) students who need documental guides in the main fields of human biology. The books in this series are intended to present scientific fact with a minimum of technical terminology and to consider the ways in which policy and action directed towards human betterment and social amelioration could stem from this.

Leff's book set a very high standard for those that were to follow. Swyer's *Reproduction and sex*, the second volume of the series, in its own way is just as excellent as was the first. The editor's choice of authors seems to be uncannily sure. Certainly there is no other book of this kind and with this particular purpose published in Great Britain that can equal this in value and in trustworthiness. It is well planned and well written. The author's qualifications for the writing of it certainly derive from far more than his specialist knowledge of endocrinology.

The book gives an accurate and up-to-date account of existing knowledge concerning human sexuality and reproduction. Common misconceptions are considered and fairly destroyed. The latter part of the book is largely concerned with the psychological aspects of sexual behaviour and is based, for the most part, on the Kinsey reports. The author displays an admirable reasonableness and objectivity in dealing with these matters which can so easily become enmeshed in prejudice and distorted by emotion. His scientific yet humane approach to the difficult, distressing and socially important problems of sexual perversions makes this a book much to be commended to all who seek enlightenment concerning them.

As I read this book with lasting pleasure and increasing profit, my mind halted twice, on pages 20 and 240. Is it really the case that, if "males who have always been deficient in male hormone [and who therefore] show characteristic deficiencies of behaviour" are given testosterone, they will undergo profound transformation, becoming capable of showing initiative and of accepting responsibility? This would seem to be implied by what is written. And has anyone actually demonstrated the existence of the cords of Pflüger?

The editor and the author have done a fine job of work. They deserve high reward. This can take one form only—that this book, being widely studied, shall mould opinion and fashion action in increasing measure. Should this happen, our society will indeed be fortunate.

F. A. E. Crew

The Biochemistry of Semen

T. Mann. London: Methuen & Co. Ltd.; New York: John Wiley & Sons, Inc., 1954. (Methuen's Monographs on Biochemical Subjects.) xiv + 240 pages; 7 plates; 16 figures. 19 × 12 cm. 16s.

The intricacies of science become more complex every day and their details are dispersed through an ocean of literature; therefore one has a special welcome for a book like this, written as it is by a leading authority who has collected and integrated a vast number of facts. Having in the first chapter described the minute anatomy of spermatozoa and dealt in a general manner with the seminal plasma, the author goes on to discuss the origin, constitution and biological effect of each component of the latter. The abundant information he supplies on all these points has been condensed into a remarkably small space by a proper economy of words and an easy explanation of their meaning. This we think is scientific writing at its best; and, seeing that a thousand references and a particularly full index are included in the 240 unusually small pages, Dr Mann has given an example that deserves imitation.

The avoidance wherever possible of technical jargon is among other merits. When a special term is used that may not be familiar to an individual working in another field—such a word, for example, as "acrosome"—perusal is made easy by a simple definition. In such a way one is made to understand the meaning of "capacitation", which to the reviewer is new and irreplaceable by any other term. Occasionally, acid phosphatase is referred to in the text as prostatic phosphatase; and this in some ways seems an improvement on current practice.

In conclusion one may say that Dr Mann has produced a "source" book of considerable value that no one seriously interested in the subject can afford to overlook.

Harold Burrows

Biochemistry of the Aminosugars

P. W. Kent & M. W. Whitehouse. London: Butterworths Scientific Publications, 1955. x + 311 pages; figures. 22 × 14 cm. £2

Ten years ago, interest in the study of the mucopolysaccharides and mucoproteins was largely confined to a very few small groups of workers in Europe and America: the position has certainly changed today. The subject quite suddenly began to appeal to the imagination of the generality of biochemists, and with equal suddenness publications in the field began an exponential increase. It is now many years since a comprehensive survey of the subject has been attempted, and the appearance of such a survey at this time of rapid development is all the more welcome.

About half the book deals with the chemistry of the amino sugars and their derivatives, and this section is exceptionally clear, thorough and well set out. In attempting critical reviews of the physiological and biochemical aspects the authors are on much more difficult ground, largely because current views are in a state of flux. However, if this is carried in mind by the general reader, the survey provides a fair view of the present state of the subject as a whole, and the specialized sections, although introductory, provide much valuable information.

The general discussion in chapter IV on the state of biological macromolecular products as found in tissues and in their isolated condition is both helpful and challenging, but too little is known about the nature of the bonds involved in complex formation for an adequate discussion of this point. For the same reason discussion of the biological activity and biochemical properties of recently recognized members of the group such as the gonadotrophic hormones is necessarily limited.

The new classification of the mucosubstances given early in the book is interesting. Terminology in this field has become rather chaotic, and some general agreement on terms at this stage would be extremely valuable. The suggestion of the authors that the use of the term "mucopolysaccharide" to describe substances which are solely polysaccharide in character (such as chondroitin sulphate or hyaluronic acid) should be discontinued, is a good one; the replacement of the old term by "aminopolysaccharide" is equally logical and unambiguous. However, many workers in the field may think it unfortunate that the authors also suggest discarding the word "mucoid". This is well established in the literature and could with advantage be retained to describe polysaccharide–peptide complexes of the "ovomucoid" type, in which the amino-acid constituents are an integral part of the molecule.

S. M. Partridge

Scientific Books, Libraries, and Collectors: A Study of Bibliography and the Book Trade in Relation to Science

J. L. Thornton & R. I. J. Tully. London: Library Association, 1954. x + 288 pages; 12 plates. 22 × 14 cm. £1 4s. (18s. to members)

This is not an exhaustive treatise on the bibliography of science; the authors describe it as an introductory history of the production, distribution, and storage of scientific literature from the earliest times. They have endeavoured to record the chief writings of every prominent scientist to the end of the 19th century, with details of the more important editions of his works. In doing so they have built up a short but extremely valuable biographical history of science.

As is to be expected in a book of only 242 pages of text, there are a number of omissions, some intentional, but apparently few of importance. The compact style of the writing does not make for easy reading, and the footnotes which abound on every page are a little irritating, particularly as they are almost all repeated in the extensive bibliography at the end of the book. Nevertheless, the authors are to be congratulated on their industry in marshalling so many facts and on their skill in presenting them in a form so easy to consult. The book obviously represents some years of hard, meticulous work. The student of the subject and the scientist trying to fill bibliographical gaps will find between its covers a wealth of material on the bibliography and biography of science. There are chapters on scientific literature before the invention of printing, incunabula, scientific books of the 16th, 17th, 18th, and 19th centuries, the rise of scientific societies, the growth of periodical literature, private libraries, publishing and bookselling, and scientific libraries of the present time. The index, adequate for personal names, would have been made still more useful by the addition of more subject entries. The plates reproduce title pages of some of the most important books and journals mentioned in the text.

The book is set in Bembo, a pleasing type face and a happy choice for a work where so much italic is required. The accuracy of the setting of a rather difficult text reflects credit both on the authors and printers. This book should prove a valuable acquisition for libraries, collectors, and booksellers. It will deserve an important place among the literature on the history of science for some years to come.

L. T. Morton

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INTRODUCTION

Surgery of the Heart and Thoracic Blood Vessels

The surgery of the heart and great vessels is a recent and very rapid development and, moreover, has attracted so much interest, often of a sensational nature, that it is in danger of being wrongly appraised. To many it is still a desperate form of therapy and, because new, still uncertain and untried.

Although successful operations within the substance and structure of the heart, apart from suture of wounds and removal of foreign bodies, date back only some seven years, they have now become so numerous and commonplace as to have assumed a secure position amongst the standard operations of surgery. It is wrong to assume that, because they are so new and so easily dramatized, they are necessarily uncertain. The very reason for their rapid acceptance and development rests upon their success: a success all the more striking and welcome because it provides help where before no help was possible. This is especially striking in the surgery of mitral stenosis. A few years ago this remained as one of the great unsolved problems of surgery; it has now brought relief of disability to thousands throughout the world and saved the lives of many who otherwise had no hope when routine medical treatment had failed. The need for mitral valvotomy, and its great success, are testified by the demand from the many sufferers for operative relief; most surgical centres throughout the world have big waiting lists.

Aortic valve surgery presents more difficulties and problems than mitral valve surgery, but much progress has been made. N. R. Barrett gives the present position in a comprehensive article. When we survey the whole field of cardiovascular surgery we realize that a great new surgical industry has evolved.

Familiarity with cardiac distress and arrest, and the prevention or treatment of these conditions, are paramount in the surgery of the heart. The article by I. K. R. McMillan deals with this important subject. It is still too little known that, far from being a delicate structure that will stop readily if touched, the heart is a tough organ which will do its best to go on beating if at all possible and, even when grossly maltreated beyond the point of distress to apparent death, it will still respond and work once more if handled properly.

The development and application of cardiac surgery is impossible without close co-operation with the cardiologist, and the articles by Maurice Campbell and by Paul Wood emphasize how much help the surgeon can obtain from his physician colleagues.

The surgery of the great vessels is as full of intimidating problems and moments as is the surgery of the heart itself. The earliest encouragement towards the surgery of congenital heart disease came from closure of the persistent ductus: a problem dealt with in a lucid manner by O. S. Tubbs, himself a pioneer in this field and holding credit for the first successful cure by operation of an infected ductus. O. S. Tubbs also deals with the surgery of aortic coarctation, which was introduced at the end of the Second World War and has developed rapidly.

Today, many more cases are being revealed as a result of mass radiography and examination for national service, and there is now fairly general acceptance throughout the world that all patients (except the elderly) should be operated upon as soon as reasonably possible after the diagnosis has been made. The surgery of aortic resection for coarctation has been rendered much more satisfactory since the introduction of vessel grafting; gone is the surgeon's fear that he may be unable to restore continuity. Even resection of a long length of aorta containing an aneurysm is now not only possible but is a standard procedure. C. G. Rob & H. H. G. Eastcott, pioneers in the establishment of the blood-vessel bank and in the development of the most modern methods of preservation of vessels, recount their large and almost unique experience in vessel grafting. Of special interest is their experience that thrombosis follows in a high percentage of cases when the limb vessels are replaced and also their experience of the inferiority of venous autografts to arterial homografts. This latter observation is particularly important. Their enthusiasm about the future of foreign materials, such as plastic cloth, is less likely to be generally acceptable until large experience and a long-term follow-up justify it.

The great contribution of cardiac surgery to cardiology dealt with in these articles is in regard to therapy, and in this, quite rightly, the true emphasis lies. However, the impact of surgery on cardiology has had other effects apart from pure therapy. It can even be said that cardiac surgery has been responsible for a much-needed renaissance in cardiology which had reached a stage of slow progress. In spite of the disparagement that is often extended to surgery as opposed to medicine (although really the two are indivisible), the influences of surgery in any field have usually proved stimulating and provocative and have led to great advance; cardiology of all the specialties has been the least exposed, hitherto, to this stimulating effect of surgical contact.

One of the big results of the advent of surgery has been a revision of the standards of diagnosis. This is not to say that there was not a high standard of diagnosis in cardiology earlier, but, now that operations are being performed, a very high standard is essential. This is well shown in congenital heart disease in which many time-worn beliefs and shibboleths have had to be discarded; in acquired heart disease the frequent difficulties in recognition of mitral regurgitation serve as another example. It is necessary to emphasize that, even with all the latest methods of scientific and radiological investigation, it may be impossible to achieve an exact diagnosis until the heart is actually exposed at operation. In other words modern cardiology extends right into the operating-theatre.

Another feature brought to cardiology by surgery is a proper regard for the importance of an obstructive process in the disordered function of an organ, and recognition of the paramount need for relieving such an obstruction. The relief of an obstruction has been one of the basic tasks of the surgeon for many centuries and he has acquired much simple but valuable knowledge of the reaction of the body to an obstruction. If this basic principle of the need to relieve an obstructed viscus is applied to the heart, many of the difficult decisions of treatment become easier. For instance, if an obstruction is present and is threatening life, the surgeon knows it is his duty to relieve it at all costs, unless the patient is *in articulo mortis*. The problems presented by unusual valve stenoses, multiple stenoses and various complicated combinations of circumstances become simpler if this is remembered. Many of the different phases of our life and work become easier if we can recognize and accept the principles that should govern our decisions and actions.

The surgery of the heart has developed so rapidly that we are in danger of not keeping up with proper progress unless the basic principles are recognized. Cardiac

surgery has so far concerned itself with "running repairs" on the intact functioning heart. Great progress has been made in this, but it is clear that we have almost reached the end of useful development in this way. It is difficult to visualize much more progress until open definitive surgery of the heart is established. All present and future policy must be tested against this. The problem of the indirect operations for the treatment of Fallot's tetralogy as opposed to the direct operations provides an example.

From the above principle it should be clear that only direct operations upon the cardiac lesion itself, in an attempt to restore normality, can be acceptable. The final total correction of the lesion depends upon safe open-heart surgery. That this is possible has been shown by the brilliant work of the Minneapolis group in the use of cross-circulation, in which the ventricular septal defect has been closed successfully in cases of Fallot's tetralogy as well as in many cases in which it was the sole lesion. This work rests on the splendid basic work done by Andreasen & Watson at the Royal College of Surgeons of England on the "azygos factor" in allowing survival with an interrupted circulation; A. T. Andreasen's article in this Bulletin is packed full of valuable and fundamental experimental observations in addition to sound reasoning on the whole problem.

Kirklin and his team at the Mayo Clinic, Rochester, Minnesota, have recently produced an artificial heart-lung machine that has allowed them to do open-heart surgery with safety. The whole subject of the artificial heart-lung and the contributions from Great Britain are dealt with by W. P. Cleland & D. G. Melrose.

Both cross-circulation and the heart-lung machine have certain drawbacks, chiefly associated with complexities and dangers. In an attempt to find a simpler method of operating on the interior of the heart under direct vision, many workers have also studied the use of hypothermia. E. J. Delorme, a pioneer in Great Britain on hypothermia, contributes an invaluable survey of the subject. D. N. Ross, in his two articles, discusses principles of hypothermia and also its application to closure of atrial and ventricular septal defects. The policy in the Thoracic Surgical Unit at Guy's Hospital has been to try to develop hypothermic methods specifically as an alternative and preferable means of open intracardiac surgery. The latest technique, not fully mentioned in D. N. Ross's paper on hypothermia, has many advantages and moreover illustrates an important principle. Our policy is to open the chest at normal temperature, examine the heart and complete the diagnosis. If then hypothermia is needed, it can be rapidly and simply achieved by inserting catheters, via the right auricle, one into the superior and one into the inferior vena cava; blood is then pumped from the superior vena cava, through a cooling coil and back into the inferior cava. After the operation within the heart is completed, the veno-venous circuit can be used for rapid warming. Even if need for hypothermia is not anticipated but is found necessary when the heart has been exposed, it is possible to institute it without delay and with little trouble. Time and further experience alone can show whether in hypothermia and its various modifications and elaborations we shall have a fully satisfactory method of open-heart surgery. Whatever the method, the future must surely lie in open definitive operations for many diseases of the heart.

Russell Brock

We are much indebted to Sir Russell Brock for acting as Chairman of the Committee that planned this symposium, and to Mr N. R. Barrett for acting as Scientific Editor.

ANAESTHESIA FOR CARDIAC SURGERY

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The recent advances in the surgery of the heart and great vessels have been permitted by the progress of anaesthesia, and surgical techniques now await the perfection of hypothermia and artificial circulation to make their next advances. The first condition for success in operations on the heart is the prevention of deterioration in the patient's condition during the period of the open pneumothorax. Thoracic surgery was sadly hampered until the technique of controlled respiration by intermittent positive pressure was introduced. Before this the only elective procedure of cardiac surgery with an established position was the decompression of the heart in constrictive pericarditis—even this was limited to an anterior approach to avoid opening the pleural cavity. Injury to the pleura was then a serious occurrence predisposing to operative and post-operative complications.

A further obstacle to the development of heart surgery in the past was the difficulty of induction of anaesthesia in cases with severe circulatory embarrassment. The second stage of anaesthesia, that of heightened reflexes, was always a time of hazard. The apprehensive patient was liable to struggle and throw an unwarrantable burden on a disordered circulation. Laryngeal spasm causing sub-oxygenation was troublesome in the healthy patient, but it was terrifying when the myocardium was so little removed from failure. An increased circulation time caused the concentration of anaesthetic in the brain to lag behind that in the air passages: laryngeal spasm was more frequent and induction more difficult. Finally there was the fear of reflex disturbance of cardiac rhythm from vagal stimulation during intubation.

The methods of anaesthesia described here have been developed against a background of these past difficulties and represent a combined experience of 800 cases involving cardiac surgery.

1. Pre-Operative Preparation

The success of the operation in all but the simplest case depends on team-work. Much of the pre-operative preparation is in the hands of the cardiologist and it is to him that the anaesthetist looks for a detailed assessment of myocardial function and of the optimum time for operation. Rest from the usual stress of life, careful attention to salt and water balance and suitable treatment with digitalis build up a cardiac reserve ample for careful anaesthesia. The first important task of the anaesthetist is to gain the confidence of the patient by explaining details of the preparation so that, as far as possible, the patient comes to operation relaxed. These subjects are particularly prone to emotional disturbance, and this may precipitate pulmonary oedema.

These patients may have pulmonary congestion despite careful preparation and they all receive pre-operative physiotherapy and instruction in breathing exercises. This teaches them to eliminate sputum more readily and to breathe more effectively—both of importance after thoracotomy when pain and stiffness cause reduced chest movement and sputum retention.

Careful attention to oral hygiene and to the presence of crowned teeth, bridgework and dentures at the pre-operative visits may save dangerous delays during the induction. This opportunity is also taken to identify veins suitable for induction and transfusion. The judicious use of sedatives for several days before operation is of great value in relief of anxiety. Finally the pre-medication is arranged.

2. Pre-Medication

Pre-medication normally serves three functions: to allay anxiety, to dry secretions in the air passages and to reduce reflex activity. Sedation is most important for these patients, for it reduces the metabolic demands of the body and so eases the burden on the heart. It serves, to some extent, as a basal anaesthetic, and smaller doses of other drugs are required during the operation. Atropine and, to a lesser extent, scopolamine used to reduce secretions have the disadvantage of causing tachycardia. A rapid heart is troublesome for the surgeon, but it is the shortening of diastole and incomplete filling of the ventricle that may cause grave circulatory embarrassment.

As a sedative we use Omnopon (10–20 mg.) or pethidine (50–100 mg.) one to one-and-a-quarter hours before operation. In children we use them in doses proportional to weight—Omnopon (2.5 mg.) or pethidine (10 mg.) per stone[1] bodyweight. In many cases this is accompanied by atropine or scopolamine, but where an undesirable tachycardia or cardiac irritability is feared, particularly in severe mitral stenosis, pethidine alone is given. This drug reduces secretion and, if reinforced by promethazine (25–50 mg.) as a sedative and antispasmodic, a comfortable and easily managed patient arrives in the anaesthetic room.

We have not found chlorpromazine helpful in these cases. The hypotension and tachycardia associated with effective dosage are undesirable during the operation, and its prolonged action after operation delays recovery of consciousness.

3. Induction

The fundamental consideration in the administration of any anaesthetic is the maintenance of gaseous exchange. Oxygen

1 stone = 6.35 kg.—ED.

depletion alone or together with carbon dioxide retention calls for a physiological response which may well be beyond the limited reserve of the subject of cardiac disease. Sub-oxygenation sets up a vicious circle: lowered cardiac output —fall in blood pressure—diminished coronary blood flow. Anoxia is also the most important predisposing cause of arrhythmia during heart surgery.

The patient on arrival in the anaesthetic room is placed in a position of minimal respiratory discomfort. If orthopnoeic, he is well propped up on pillows. The distress, physical and mental, of lying flat may well precipitate pulmonary oedema.

The pulmonary congestion or incipient pulmonary oedema of many of these patients interferes with transference of gases between the alveoli and blood stream; minor disturbances of ventilation caused by straining, coughing, laryngeal or bronchial spasm or the presence of bronchial secretions may rapidly lead to disaster. We like to give oxygen for several minutes before the induction: a smoother induction follows and more time is available for intubation if a high oxygen concentration has been maintained.

Some form of topical analgesia to the larynx has proved helpful before intubation. In severe lesions it is an advantage to achieve this before starting the anaesthetic and so to eliminate the possibility of spasm; but in straightforward cases it is more pleasant, for the patient, to wait until he is asleep. Lignocaine (Xylocaine), 4%, has proved very satisfactory when given by cricothyroid injection or by laryngeal spray.

All anaesthetic drugs cause vasodilatation and may be associated with a fall in blood pressure. Many of these patients are unable to increase their cardiac output to compensate for a reduction in peripheral resistance, and coronary blood flow is diminished. Any drugs used are given very slowly and in minimal dosage to avoid these undesirable effects.

In the majority of cases we use intravenous thiopentone for induction. As the total dose is seldom more than 300 mg. it is most conveniently given in 2.5% solution. The prolonged circulation time in many of these cases leads to delay in the injected drug reaching the brain and this may be mistaken for an apparent increase in tolerance. An unnecessarily large dose may be given if a more concentrated solution is used.

It is of interest that the orthopnoeic patient will tolerate the horizontal decubitus as soon as consciousness is lost: a result of the reduced venous return to the right heart together with the abolition of the psychic factor.

We prefer to use intravenous pethidine in doses of 20 mg. in resistant cases rather than to increase the doses of barbiturate. As soon as the patient is comfortably asleep, a muscle relaxant is given. We use tubocurarine chloride (15–30 mg.). Oxygen is given continuously, at first by assisted, and later by controlled respiration, until the full effect of the relaxant is developed—after at least 3 min. A large-bore cuffed endotracheal tube is then passed under direct vision. In children we use the largest uncuffed tube that will pass comfortably through the larynx.

Positioning the patient upon the operating-table is now carried out slowly and gently. The changes in the haemodynamics required by a change of posture are made with difficulty—there is usually a fall in blood pressure which may be severe if the movement is too rapid.

The next step in the anaesthetic technique is the insertion of a paravertebral block, with 0.5% lignocaine, of the segments corresponding to the intercostal incision, and two spaces above and two below. This block for convenience is made by the surgeon. It serves two useful purposes: it reduces the quantity of general analgesic drugs needed to keep the patient free from reaction and it keeps him free from wound pain in the first post-operative hours, allowing deep breathing and coughing. We usually have 1: 300,000 adrenaline in the local solution, a practice which is open to the criticism that it increases the irritability of the heart. If worried by the patient's condition, we omit the adrenaline, but in many cases this amount of adrenaline does no more than check the fall in blood pressure caused by moving the patient into position.

4. Controlled Respiration

We consider the complete control of respiration to be essential in all but the simplest of cases. If the dose of curare used in the induction is insufficient to paralyse respiration completely, a further dose is given before the operation is begun.

Spontaneous respiration in the presence of an open pneumothorax is accompanied by a progressive deterioration in respiratory efficiency, owing to the pulmonary collapse, paradoxical movement and mediastinal " flap ". Ever-increasing efforts are made by the healthy patient to overcome these difficulties, as a result of the increasing stimulation of carbon dioxide retention and oxygen lack. The cardiac invalid is incapable of making these additional efforts and would rapidly pass into respiratory failure.

Assisted respiration is contra-indicated for a number of reasons. Firstly, the frequency of respiration is dictated by the patient, and this may cause unwanted respiratory movement during difficult phases of the surgery. Secondly, for the anaesthetized and partially paralysed patient to make respiratory movements, a lowered oxygen and raised carbon dioxide level is implied. These are factors which predispose to cardiac arrhythmia. Thirdly, if assisted respiration is efficiently performed, it overcomes the state of under-ventilation, and controlled ventilation takes its place. Fourthly, assisted respiration precludes the use of deep curarization to reduce the amounts of depressant and undesirable anaesthetic drugs that are otherwise needed. The protagonists of assisted respiration point out that venous return is promoted by the negative intrathoracic pressure caused by spontaneous inspiration; but this is lost as soon as the pleura is opened. Greater positive pressures may be required to assist ventilation in a partially curarized patient than to control ventilation in a completely paralysed patient. An inspiratory pressure of 20–25 cm. of water is often necessary. An expiratory pause longer than the inspiratory phase is essential with these pressures to allow adequate deflation of the lungs between breaths. With such pressures, and in patients with the lung changes associated with chronic heart disease, the exposed lung tends to push forward into the site of operation and has to be restrained by packs and retractors. It is unwise to allow the lung to collapse completely to facilitate surgery, as this may reduce the available respiratory surface below a safe level.

One of the advantages of controlled respiration is that periods of apnoea to assist the surgeon in difficult manoeuvres can be tolerated, providing that full ventilation is carried out at other times.

5. Maintenance of Anaesthesia

After intubation, controlled respiration is continued by using a mixture of 40% oxygen and 60% nitrous oxide

delivered into an absorber circuit. To maintain a constant percentage despite losses from the circuit, a total flow of 5 l./min. is added and the excess allowed to escape through a leak so adjusted as to keep the rebreathing bag comfortably filled.

The 60% concentration of nitrous oxide maintains unconsciousness indefinitely in patients who have been pre-medicated and in whom anaesthesia is induced in the manner described. This light level of anaesthesia minimizes vasodilatation and aids a rapid post-operative recovery. Sub-oxygenation occurring with this mixture is very suggestive of under-ventilation. Although higher oxygen tensions can be used, they allow a patient to be well oxygenated but hypercarbic: the full value of the nitrous oxide is lost.

For many short procedures no further drugs are administered. In resistant patients and in operations lasting more than an hour and a half additional drugs may be necessary.

a. Indications for Additional Relaxant

These are: (i) an increase in the inspiratory pressure needed to maintain tidal volume; (ii) an increased tendency for the lung to billow into the wound, owing to decreased expansion of the other lung; (iii) return of spontaneous respiratory movements usually heralded by jerky diaphragmatic movement. Also, before commencement of arterial suture where sudden movement might jeopardize the success of the operation, an additional dose of relaxant is frequently advisable if some time has elapsed since the previous dose.

The end-point of the action of curare is indefinite and a further dose of relaxant can be avoided towards the end of the operation by over-ventilation which removes the stimulus of carbon dioxide.

b. Indications for Additional Analgesic

These are: (i) movement of the fine muscles of face or hand; (ii) increase in pulse rate and fall in blood pressure not explained by the surgical manipulations at the time; (iii) dilatation of the pupil; (iv) secretion of tears; (v) vasoconstriction evidenced by pallor, drop in skin temperature and delayed capillary refill; (vi) sweating; (vii) the onset of bronchospasm (slight increase of resistance to inspiration, and prolongation of expiration) if the presence of sputum in the air passages can be excluded.

The drug we have used for this purpose has been pethidine in doses of 20–25 mg. This has been repeated as necessary but in practice is seldom required more than twice.

c. Indications for Additional Thiopentone

Bearing in mind the cardiovascular depressant actions of thiopentone, we have avoided using this drug after the induction as far as possible. If the signs indicative of more pethidine do not resolve after it is administered, then a small dose of thiopentone (e.g. 50–100 mg.) is given. The signs of too light a level of analgesia are in many cases identical with those of commencing shock. This condition must be excluded before extra thiopentone is contemplated.

d. Untoward Incidents

Emboli. In operations for the relief of mitral stenosis, clot may be found in the auricle and its appendage, and a grave risk of embolism is present. To reduce the danger of cerebral embolism, the carotid arteries may be compressed for short periods either manually in the neck or by means of clamps or tourniquets within the chest. This compression can be maintained safely only for short periods but may even then be associated with carotid sinus reflexes. Small doses of hexamethonium (10 mg.) have been used to block these reflexes, and the clinical impression has been gained that this is valuable. No hypotensive effect is noticeable with this small dose.

The occurrence of cerebral emboli can frequently be diagnosed by the anaesthetist. Sweating, a sharp fall in blood pressure which does not return to its former level, a deterioration in colour despite adequate ventilation, and deep unconsciousness at the end of the operation when most patients are awake, are suggestive. Peripheral emboli may show themselves by loss of pulse in a limb, but this is not usually noticed until the end of the operation. The rapid return of consciousness in the theatre of these patients allows simple instructions to be carried out by the patient in order to establish whether cerebral embolus has occurred. Palpation of the peripheral pulse is also carried out as a routine. The anaesthetist can assist in such cases by performing the appropriate sympathetic block, e.g. a stellate block for cerebral embolus.

Arrhythmia. Surgical manipulation is the main cause of cardiac irregularity developing during operation, but anoxia and hypercarbia are of almost equal importance. Direct stimulation of the heart causes many bizarre patterns on the electrocardiogram. Recognizable arrhythmias include multiple auricular extrasystoles, sinus bradycardia, nodal rhythm and heart block. These irregularities usually disappear rapidly on cessation of stimulation. The more sinister arrhythmias such as multifocal ventricular extrasystoles which may be the precursor of ventricular fibrillation usually have some further excitatory causes, of which anoxia, use of vasopressors, atropine and *cyclo*propane are the most important. Insufficient and too great depth of anaesthesia are causes of irregularity, as are also laryngeal and bronchial reflexes.

We have abandoned the use of intravenous procaine and procaine amide as a routine in the prevention of these disturbances. A dose sufficient to decrease cardiac irritability causes vasodilatation and a marked fall in blood pressure; the accompanying myocardial depression increases the difficulties caused by this fall. We feel that the most important prophylactic measures are a meticulous attention to secure proper ventilation and the avoidance of drugs which are known to increase myocardial irritability.

Cardiac arrest. The emergencies of cardiac standstill and ventricular fibrillation are dealt with elsewhere in this symposium.[2]

Efficient ventilation of the lungs with pure oxygen is continued, and the effectiveness of the cardiac massage can be assessed by palpating the carotid artery. It is important in feeling for carotid pulsation that no obstruction is caused to the cerebral circulation by continued pressure of the fingers. The artificial respiration and artificial circulation are maintained until the restoration of cardiac function. Compression of the descending aorta may hasten the recovery of spontaneous heart beat by diverting more of the blood flow through the coronary arteries. This redistribution can be assisted by small doses of Methedrine (5–10 mg.) given intravenously except in cases of fibrillation. Cardiac standstill usually responds to massage and the intraventricular injection of adrenaline, unless it is the result of exhaustion after long-continued oxygen lack. Ventricular fibrillation calls for the injection of procaine (100 mg.) or lignocaine (50 mg.) into the heart, and possibly electric shock may be needed to stop the fibrillation.

Sudden haemorrhage. Sudden haemorrhage calls for imme-

[2] See McMillan, p. 229 of this number of the Bulletin.—Ed.

diate replacement of an equal volume of blood by transfusion, and we prepare for this by giving an intravenous transfusion in all cases. When cardiac arrest follows severe bleeding, the intravenous transfusion sometimes fails to produce sufficient filling of the heart to allow effective massage. A rapid intra-aortic transfusion may raise the aortic pressure enough to produce some perfusion of the coronary arteries.

6. Restoration of Spontaneous Respiration

At the conclusion of the intrathoracic procedure the lung is re-expanded, by prolonging the inspiratory phase of respiration. If the presence of any excessive secretion in the bronchi is suspected, it should be aspirated before this expansion is attempted. Sometimes small areas of lung resist these attempts. Gentle stroking of the lung at these points is frequently successful in allowing aeration of the patches, suggesting that some form of local bronchospasm is responsible for the failure of inflation; we have occasionally given intravenous atropine at this stage to assist re-expansion.

When the chest has been closed after full expansion of the lung the carbon dioxide absorber is removed from the circuit. Over-ventilation during this period of re-expansion may well wash out an excessive amount of carbon dioxide. Until the chest is closed this is advantageous, for it ensures that there will be no spontaneous respiration to form a pneumothorax by drawing air into the partially closed wound. Once the chest is closed the normal stimulus to breathing should be restored as soon as possible.

We use neostigmine (1–2.5 mg.) to overcome the residual action of the curare, as soon as the operation is complete and we have some evidence of the return of muscle power. The neostigmine is always preceded by intravenous atropine (0.5–1 mg.). The rise in pulse rate following the atropine signals that it is safe to give the neostigmine. If this does not restore adequate respiration, some other cause of respiratory depression is sought. When the respiration is adequate in depth and frequency, the nitrous oxide is discontinued and oxygen is given. The endotracheal tube is removed after pharyngeal and tracheal suction to remove accumulated secretions. Consciousness is very quickly regained. The risk of a high oxygen concentration disguising a retention of carbon dioxide should be kept in mind, but this is unlikely if the respiration is apparently normal. Carbon dioxide is more readily excreted through congested lungs than oxygen is absorbed, so that the use of high percentages of oxygen in the immediate post-operative period is valuable.

In most of these cases a pleural drain connected to a water seal allows the escape of air or fluid from the pleura. It is important that the fluid level in the water seal should be swinging freely with respiration, as the development of a pneumothorax or haemothorax could be rapidly fatal in some of these patients who have little reserve.

Post-operative pain is controlled by small doses of pethidine (50 mg.) or Omnopon (10 mg.). Care is taken to avoid respiratory depression; sub-oxygenation is itself a potent cause of restlessness and predisposes to circulatory collapse.

7. Specialized Techniques

There are two simple modifications of the technique described above. Patients with constrictive pericarditis sometimes tolerate thiopentone badly. It is suggested that cardiac tamponade may be set up by the loss of tone of the heart muscle in a rigid pericardium. In these cases, in order to avoid this danger we use an induction with nitrous oxide, oxygen and ether after pre-medication with Omnopon and scopolamine and topical analgesia to the larynx. As soon as the stage of surgical anaesthesia is reached, intravenous tubocurare is given. The respiration is assisted until the relaxant has worked, when intubation under direct vision will be easy. In nearly every case we find that we can abandon the use of ether after the induction, the patient being maintained on nitrous oxide, oxygen and curare supplemented when necessary by pethidine, thus permitting the use of diathermy during the operation.

Some small children have become frightened of needles during preliminary investigations such as angiocardiograms, and for these it is kinder to produce some clouding of consciousness by the inhalation of nitrous oxide and oxygen before giving the intravenous drugs.

These methods suffice for the operations where no significant interruption of the circulation to vital organs is expected. Techniques for dealing with circulatory interruption by hypothermia or by mechanical circulation are the subject of other papers.[3]

8. Hypotension

In the majority of cases the use of hypotensive techniques is neither necessary nor desirable. Bleeding from the wound edges is not so severe as to warrant its use, and bleeding from the heart and great vessels is not controlled by its action. Hypotension is helpful in operations for the relief of coarctation of the aorta. The collateral circulation to the lower half of the body increases the vascularity of the chest wall, and any reduction in blood pressure can materially assist the surgeon and reduce the blood loss during the opening of the chest. The aorta and the intercostal arteries are pathologically fragile and may be aneurysmal. Any reduction in blood pressure makes their dissection and the preparation of the field for suture or grafting much safer.

When there is an obstruction to the flow of blood into the descending aorta, ganglion blockade may not produce the dramatic fall in pressure that can be obtained in normal subjects. Indeed care must be taken to time the dosage of blocking agents so that the hypotension is wearing off when the aortic clamps are removed and blood is allowed to flow freely. If it is still intense the opening of vascular fields in the abdomen and legs may cause circulatory collapse. We use hexamethonium, either 30–75 mg. of the bromide, or 50–100 mg. of the iodide. It is difficult to maintain a steady pressure in cases of hypotension with the short-acting agents.

The blood pressure in the right arm, head and neck may rise dangerously above the pre-operative level when the aortic clamp obstructs the left subclavian artery. The simple manoeuvre of connecting the ascending and descending aorta by a short length of wide-bore plastic tubing with a blood needle at each end has proved effective.

When the clamps are removed the administration of Methedrine may be necessary, but usually compensation occurs spontaneously in a few minutes, and by the time that the thorax is closed the blood pressure has returned to normal levels. These patients must be moved with great care and should be nursed flat for some hours until all signs of vasomotor instability have disappeared.

[3] See papers by Delorme (p. 221) and Cleland & Melrose (p. 236) in this number of the Bulletin.—Ed.

SELECTION OF PATIENTS FOR SURGERY IN CONGENITAL HEART DISEASE

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Patients with congenital heart disease may be selected for operation to relieve their present disability or to improve their future outlook though they have few symptoms at the time. Most patients with cyanotic disease belong to the former group, and most but not all of those with a patent ductus and coarctation belong to the latter group, when a much lower operative mortality is essential if operation is to be widely accepted.

One cannot, at present, expect final answers to many of the questions raised in this article. It is only 15 years since the start of surgical treatment, and operations that are hazardous today may be relatively safe within a few years. There is now wide agreement that nearly all patients with a patent ductus should have it closed—and this is a good example of preventive medicine, for the patient is cured. The conditions under which operation should be advised for Fallot's tetralogy are widely agreed, but here the patient is improved but not cured, and the improvement will not last indefinitely—perhaps only for 10 or 15 years. There is less agreement about the surgical treatment of coarctation of the aorta, because the risk may seem too great to be taken in a patient who is so fit, though a successful operation would greatly improve the outlook. These are some of the general considerations that must govern the selection of patients for operation.

1. General Principles of Diagnosis

The advances in the knowledge of congenital heart disease depend on renewed clinical study and on what has been learnt from cardiac catheterization, angiocardiography, and surgery. They have led to better understanding of the haemodynamic effects of many anatomical defects and to the establishment of some simple underlying principles as well as a mass of detailed knowledge.

There are four important general principles. (i) Central cyanosis indicates a right-to-left (veno-arterial) shunt. (ii) Pulmonary arterial branches that are easily seen and pulsatile indicate an increased blood flow to the lungs (pleonaemic lungs) and a left-to-right (arteriovenous) shunt. (iii) When the blood flow to the lungs is diminished (oligaemic lungs) the patient can generally be helped by surgery. (iv) Whether the blood flow to the lungs is increased or diminished can generally be recognized by radioscopy (Taussig, 1947).

Central cyanosis. The recognition of central cyanosis is therefore most important. Peripheral cyanosis does not indicate heart disease, though it is common in those with mitral or pulmonary stenosis and equally in many normal children on a cold day: it may be seen in the lips as well as in the cheeks and hands. Central cyanosis is best recognized in the tongue and mucous membranes of the mouth and in the conjunctivae. Clubbed fingers are added evidence, and in a small infant localized cyanosis of the nails when the hands are warm is an important early sign. The red-cell count is often eight million, and the haemoglobin reading, on the Haldane scale, averages nearly 140%. This well-known picture of morbus caeruleus includes all those with a right-to-left shunt though it is not always possible to add a full anatomical diagnosis.

Central cyanosis generally means an opening between the two sides of the heart, and something else that raises the pressure on the right side above that on the left. The most common cause is pulmonary stenosis, and the second is pulmonary hypertension: it is essential to distinguish these, for surgical help can often be given to the former but not to the latter.

The blood flow to the lungs. Whether the blood flow to the lungs is increased or diminished is generally best settled by radioscopy, though when there is much change it is obvious on the radiogram. After assessing the patient's disability, this is often the most important part of the examination in cyanotic cases. Lung fields that look unduly light, and pulmonary arteries that are small and difficult to see in their ramifications, are evidence of a diminished blood flow to the lungs (oligaemic lungs). On the other hand, lung fields that are unduly dense, with large arterial branches spreading towards the periphery, with dense blotchy shadows of the same branches seen in section, and especially with expansile pulsation that can be seen on radioscopy, indicate an increased blood flow to the lungs (pleonaemic lungs). This is very common with acyanotic cases but is found with only one or two varieties of cyanotic cases. This distinction is often but not always easy, and angiocardiography or cardiac catheterization may be needed to get an answer. It can be difficult even in pulmonary atresia (see section 2c).

Incidence of various defects. The many congenital abnormalities tend to occur in certain patterns, and the relative frequency of these helps one to know what to look for. Fallot's tetralogy is much more predominant than any one acyanotic type and is responsible for 66% of all the cyanotic cases if those with pulmonary atresia (9%) are included. The next largest group comprises those with transposition of the aorta and pulmonary trunk (8%); the proportion among infants is much larger but the early mortality is higher. The third largest group—an important one from the surgical point

of view—consists of those with simple pulmonary stenosis with a closed ventricular septum and a right-to-left shunt through a defect of the atrial septum (6%). This is followed by Eisenmenger's complex (6%), tricuspid atresia (5%), dextrocardia or isolated laevocardia (5%), and finally those who are not easily classified (4%). The incidence of the various defects among the acyanotic cases will be dealt with later.

2. Cyanotic Cases

When any cyanotic patient is seen, the first step is to decide whether the cyanosis is central or not and whether the blood flow to the lungs is diminished. If it is diminished the patient can probably be helped by surgical treatment, but not if it is increased, and so this principle is sometimes more important than a full anatomical diagnosis.

The outlook for many of these children has been completely changed by the introduction of surgical treatment. Whenever there is much disability operation should be advised. In infants, however, even when there are frequent cyanotic attacks with unconsciousness, operation should, if possible, be deferred until three, or better until five years of age, as the anastomosis generally becomes too small as the child grows. If any child over the age of three is losing ground and is unable to walk as far as before, it should be done quickly, as such deterioration is generally progressive. From five to ten years of age is perhaps the ideal time, though the results seem nearly as good in older children and it is only in those over 20 that the risk seems greater and the chance of a good result less. Operation should rarely be advised in those over 20 if they can earn their living, though they are often anxious for it: this does not apply to patients with simple pulmonary stenosis.

a. Fallot's Tetralogy

This diagnosis can be made when cyanosis has been present from birth or from 18 months of age; when there is a systolic murmur, often with a thrill in the pulmonary area, and a normal or diminished pulmonary second sound; when there is no diastolic murmur; when the heart is not much enlarged; when there is no great prominence of the pulmonary arc (though in some this border may be prominent from dilatation of the infundibulum beyond the stenosis, and less than half show a real sabot-shaped heart); and, most important of all, when the density of the lungs on radioscopy is lighter than usual. Four-fifths of the patients have the habit of squatting, but this is not confined to those with Fallot's tetralogy. These criteria should enable the diagnosis to be made with confidence. If, on the other hand, the pulmonary second sound is much increased and booming or widely split if it is palpable, the patient probably has an increased blood supply to the lungs and a raised pulmonary arterial pressure, so cannot be helped by operation. Angiocardiography and cardiac catheterization are not needed in most cases. The former will always demonstrate the right-to-left shunt, but this will already have been diagnosed. At one time we used it to try to decide if the stenosis was valvular or infundibular, or both, but when this information is needed for a direct operation it is better obtained by pressure measurements at the time.

Good results can be obtained in 70% with either an anastomotic or a direct operation (Campbell & Deuchar, 1953; Campbell, Deuchar & Brock, 1954). In experienced hands, the mortality is no more than 5% with anastomotic operations but it is generally higher with direct operations.

A good result means a great deal: the patients may be able to walk a mile[1] or more instead of 100 yards[2], and get about all day almost normally and go to school or earn a living; the cyanosis and polycythaemia quickly diminish. It is important for the parents to realize that the heart condition has not been cured and that, although the outlook is much improved, its duration is still uncertain. The blood flow to the lungs has been increased but the septal defect remains and the right ventricle is still working against the systemic pressure, whether the operation is an anastomotic or a direct one.

The patient cannot, therefore, lead a more active life without more work for the heart. The increase in the size of the heart in the first few months is generally not great and, at any rate for five or six years, it is rarely progressive or serious. The better the clinical result, the more the physician should see that the patient keeps his activity within reasonable limits. Until the ventricular septal defect can be closed, the patient has a limited outlook and increasing right ventricular strain will follow some day, but fortunately in most cases not for many years. There is no great risk of the high right ventricular pressure being transmitted to the pulmonary artery and producing a condition similar to Eisenmenger's complex after direct operations, for catheterization shows that the stenosis has been relieved rather than abolished, and the pulmonary arterial pressure does not rise much in spite of the increased flow.

b. Simple Pulmonary Stenosis with a Right-to-Left Interatrial Shunt

At first these patients are acyanotic but, as the pressure on the right side rises, a right-to-left shunt often develops and they become cyanotic, most often in the teens or twenties. As, by definition, the ventricular septum is closed, the shunt is through the atrial septum, generally through an unsealed foramen ovale but sometimes through a true atrial septal defect. The stenosis is nearly always valvular. The only surgical treatment is pulmonary valvotomy, and the general clinical picture and the selection of patients for surgery is so similar in the cyanotic and acyanotic cases that this is considered in section 3a.

Fallot's tetralogy and simple pulmonary stenosis are the two conditions in which operation is safest and most certain to be followed by a good result. Their recognition is, therefore, of great importance. In the conditions that follow, a higher degree of disability is necessary to make operation advisable because the risk is much greater, with an operation mortality of at least 20% and with a much smaller chance of a really good result. Generally an anastomotic operation is the only one that can be considered.

c. Pulmonary Atresia

It has been customary to include cases with pulmonary atresia as coming within Fallot's tetralogy, but their surgical treatment is so different that it is important to distinguish them. Although all the blood flow to the lungs is through collateral vessels, these are sometimes so numerous that they give a false impression of increased density, but the individual shadows are small and do not show expansile pulsation.

[1] 1 mile = 1.6 km.—Ed.
[2] 1 yard = 0.9 m.—Ed.

If a cyanotic patient has the general picture of Fallot's tetralogy with a continuous murmur, such as is heard with a patent ductus, he probably has pulmonary atresia and a blood supply to the lungs through a persistent ductus or through hypertrophied bronchial arteries. In other cases, this is through a large number of arteries that are too small to produce such a continuous murmur. The diagnosis is suggested by a loud single second sound (aortic) heard well over to the left in the pulmonary area (because the pulmonary trunk is small or absent), unusual concavity of the pulmonary arc due to the same cause, and a characteristic pattern of small isolated shadows of many pulmonary vessels that may show well in the upper right side of the mediastinum and round both hila, and below the aortic arch in the second oblique position on radioscopy and on angiocardiography.

Atresia of the pulmonary arteries as a whole would prevent any anastomotic operation, but generally they are patent beyond the atresia. Radioscopy can often decide if they are of adequate size for an anastomosis, but angiocardiography is often needed and should always be carried out unless one can be certain about the pulmonary arteries without it. When the lungs are dependent for their whole blood supply on arteries that arise from the aorta, it may seem illogical to add another pathway such as a subclavian-pulmonary anastomosis, but these collaterals do not hypertrophy sufficiently, and a further blood supply may help greatly.

A higher degree of disability must be used as the indication for operation than in Fallot's tetralogy, for the operation risk is more than doubled and the chance of a good result is halved. Atresia generally means that there is a thick fibrous obstruction for 1 cm. or 2 cm. and only rarely a simple valvular diaphragm, so only anastomotic operations are possible; and they are more difficult for the surgeon, as the pulmonary artery is often of poor material with thin, friable walls.

d. Tricuspid Atresia

If there is the general picture of Fallot's tetralogy but with hypertrophy of the left ventricle on radioscopy and in the electrocardiogram, the probable diagnosis is tricuspid atresia. The pulmonary trunk may arise from a small right ventricle that can be reached only through a ventricular septal defect: in these patients, as well as in those where there is pulmonary stenosis, the blood flow to the lungs is diminished, and such patients can be helped by operation. If, on the other hand, the pulmonary trunk and the aorta both arise from the left ventricle, the pulmonary blood flow will be increased, at any rate until a high peripheral resistance has developed, and the patient cannot be helped by operation. Cardiac catheterization does not as a rule give much information (unless the catheter proves that the diagnosis is wrong by passing from the right atrium to the right ventricle) but angiocardiography is often useful.

The surgical treatment is subclavian-pulmonary anastomosis. The operation mortality is no greater than that for Fallot's tetralogy but the chance of a lasting success is less, for there is a greater risk of the patient developing too much enlargement of the heart or even congestive failure.

e. Dextrocardia and Isolated Laevocardia

When the heart is on the right and the abdominal viscera normally placed (isolated dextrocardia) or when the heart is normally placed and the abdominal viscera transposed (isolated laevocardia), the heart is nearly always very abnormal, and sometimes this is so when there is complete situs inversus. The congenital defects are very complex: large atrial and ventricular septal defects, a single atrium or a single ventricle, complete or partial transposition of the aorta and pulmonary trunk, and pulmonary stenosis or atresia are all common.

f. Other Complex Abnormalities

In a cyanotic patient who does not seem to fit into any of the groups described, there is probably a complex mixture of abnormalities.

In these patients, as well as in those with dextrocardia and isolated laevocardia, operation can rarely be advised without full investigations by angiocardiography and cardiac catheterization. Though it may sometimes be successful the result is much more uncertain, so a greater degree of disability must be demanded. The risks are much greater than in Fallot's tetralogy, with an operation mortality of over 20%, and the chance of a good result much less. Subclavian-pulmonary anastomosis is nearly always the choice though, exceptionally, pulmonary valvotomy may prove possible.

Cyanotic cases with an increased blood flow to the lungs. Some operations have been devised to help cases of transposition of the aorta and pulmonary trunk but so far they have not been very successful except in a few cases. Up to the present we have not thought operation advisable in this condition.

If it becomes possible to close ventricular septal defects, operation may be possible for cases of Eisenmenger's complex, but generally the pulmonary hypertension will have been present for a long time and may have become irreversible.

3. Acyanotic Cases

There are six main conditions to be considered: three with left-to-right shunts—atrial septal defect, ventricular septal defect, and patent ductus arteriosus; and three with obstructive lesions—simple pulmonary stenosis, aortic stenosis, and coarctation of the aorta. All these are common, the first four each forming about one-fifth of all acyanotic patients, and the last two—aortic stenosis and coarctation of the aorta—forming the other fifth.

In the acyanotic group as a whole, angiocardiography is not of great practical importance. In simple pulmonary stenosis and in the three conditions with left-to-right shunts, cardiac catheterization will nearly always give valuable information in assessing the severity of the condition, though it is not needed as a routine, and operation for patent ductus can generally be advised without it.

a. Simple Pulmonary Stenosis

This is defined as pulmonary stenosis (almost always valvular) with a closed ventricular septum. The diagnosis can generally be made when there is a systolic murmur and thrill, maximal in the pulmonary area, with a pulmonary second sound that is not increased. If there is nothing else and particularly if there is no right ventricular preponderance in the electrocardiogram, the stenosis is of little importance at the time. Even when the heart as a whole is not enlarged, some prominence of the right ventricle on radioscopy and a prominent pulmonary arc, with dilatation that often spreads to the left pulmonary artery, are early signs: the pulsation that may be seen in this dilated artery can, however, be

misleading. Wood (1950) has emphasized the importance of the *a* wave in the jugular pulse and this means a moderately severe stenosis. A giant *a* wave and a large heart are late signs, and then the pulmonary second sound may be faint and the thrill may be absent.

Many of these cases are acyanotic but, as the pressure on the right side rises, a right-to-left shunt may develop if the foramen ovale is not sealed. Thus, cyanosis may develop at any age but often not till the patient is nearing or past 20, while in Fallot's tetralogy it generally starts in infancy.

Cardiac catheterization is specially useful if there is any doubt about the need for operation, for it will measure the pressure gradient across the pulmonary valve. Empirically, we have taken a right ventricular systolic pressure of 100 mm. Hg or more as an indication for operation, but this may need revision when more patients have been followed after catheterization. Operation should be considered if the symptoms are severe enough to justify it, if the heart is enlarged, or if the right ventricular strain in the cardiogram is increasing.

The only surgical treatment is pulmonary valvotomy, as subclavian-pulmonary anastomosis tends to be followed by congestive failure. Provided the patient has not been left too long, the operation risk is no more than 5%, and 80% should get good results (Campbell & Brock, 1955). The pulmonary obstruction is not cured but is reduced enough to relieve the patient of most of his symptoms, and to lower the right ventricular pressure to a half or a third of what it was before; generally a large heart becomes smaller and right ventricular strain becomes less. In the cyanotic cases, the cyanosis will be greatly improved and generally abolished.

b. Aortic Stenosis

This can be diagnosed when there is a rough systolic murmur and thrill in the aortic area. There is generally some prominence of the left ventricle and of the first part of the aorta on radioscopy; but a small pulse, an absent aortic second sound, and enlargement of the heart are late signs. The general picture is parallel to that of pulmonary stenosis, except that the patients cannot develop a right-to-left shunt and are more prone to anginal pain and syncopal attacks on exertion. Many patients do well for years until atherosclerosis and calcification make the obstruction more severe. Operation is less satisfactory than for pulmonary stenosis because regurgitation is more likely to occur and is more serious when it does, but it should be considered when there are increasing symptoms and especially when the cardiogram shows progressive left ventricular strain.

c. Coarctation of the Aorta

The diagnosis is easy if one remembers to feel the femoral pulse in every patient with high blood pressure in the arms, unexplained aortic incompetence, or striking pulsation in the neck. Most young patients will do well for 20 years but relatively few will be as fit as they would wish when they are 40, and even before this there are serious risks of congestive heart failure (30%), of rupture of the aorta (15%), and of cerebral haemorrhage (15%) from the combination of congenital berry aneurysms and the high blood pressure. With this prognosis, operation may seem advisable in every case, but it is difficult and has a considerable mortality, up to 10% even in the hands of experienced surgeons.

My present practice follows these general lines. If a patient has reached 25 or 30 years of age without symptoms or progressive signs, he is likely to do well and needs no treatment. If a younger patient is doing well, he or his parents (if he is under 18) are told that he is far more certain to be in good health at 40 after a successful operation than without it, though it carries considerable risk. Rather to my surprise, this information is enough for many patients, but fewer parents, to decide that they wish to have it done. Otherwise I am influenced by the patients' progress and often wish to see them for one or two years before deciding. There is a natural tendency for the blood pressure to rise as the child grows, up to 16 or 18; but if it continues beyond this age or if it is unusually high; if the left ventricle is much enlarged; or if there is left ventricular strain: the patient should be urged more strongly to have an operation, though he or his parents should know of the risk. It should be advised more readily in boys and young men, as the natural prognosis is better in girls.

Operation can lead to a good reduction in the blood pressure, in many cases to a normal level. This must make cerebral haemorrhage less likely, but one cannot be certain that it will abolish this risk. When a graft has to be used, which may be in one-tenth of the patients, there must be some uncertainty about the future.

A special problem arises from the frequent presence of aortic incompetence. Occasionally this may be due to rheumatic heart disease; more often it is due to sclerosis of bicuspid aortic valves, or more rarely to dilatation of the aortic ring from the high pressure. Aortic incompetence, like coarctation, is throwing an added strain on the left ventricle, and their relative importance must be taken into account in advising about an operation.

The final answer about the surgical treatment of coarctation of the aorta will need very thorough follow-up studies for the next 20 years both of those who have, and of those who have not, had an operation.

d. Patent Ductus Arteriosus

Here there is little difficulty in selecting patients for surgical treatment. The diagnosis is easy and can be made in 95% of cases by the characteristic murmur. The degree of arterial pulsation, the pulse pressure, the size of the heart, and the loudness of the murmur will help to assess roughly the amount that is going through the ductus. Enlargement of the left ventricle on radioscopy and of the aorta and pulmonary trunk may help, but the right ventricle should be normal. The electrocardiogram does not help in the diagnosis but is important, as any degree of right ventricular preponderance suggests the presence of complications and the need for full investigation before operation. Left ventricular strain is an urgent indication, but in the future such patients should have been operated on earlier, for it is generally a late development.

Cardiac catheterization is not often needed but should always be carried out when the diagnosis cannot be made with confidence and yet there seems a chance that there may be a patent ductus: this applies especially to young children who have large hearts or are doing badly, where the continuous murmur may not have developed or may be prevented by pulmonary hypertension. It should also be performed where there is any right ventricular preponderance to see whether this is due to the presence of other complications or to pulmonary hypertension alone. In the former case, operation must nearly always be avoided, as the ductus is likely to be carrying blood to lungs that have a diminished flow. In the latter case, operation is urgent as the pulmonary hypertension

is generally reversible and there is no way of knowing that it is not so in any particular case, though the longer it has been left the more doubtful this must be. If the pressure is high enough to cause a balanced or, more rarely, a reversed shunt, the result of operation is uncertain, but operation should be considered at any rate in small children.

Nearly all patients with an uncomplicated patent ductus should have surgical treatment to close this. The only exceptions are those where the flow is so small or the murmur so insignificant that one is not confident of the diagnosis without catheterization: this will include a few where the ductus is very small or is, perhaps, closing, and will hardly be one case in twenty. There is no object in waiting, on the chance that the ductus may close spontaneously, for this is rare.

Any age up to 16 is equally suitable for operation, though the longer the condition is left the more doubt there is about a perfect recovery with the heart and pulmonary artery returning to normal size. From 16 to 25 or 30 years of age, operation should generally be advised, but there is a greater risk—which increases with the years—that the condition of the arteries will make it less easy. By 30 or 40 years of age, a higher proportion of patients may be left without operation but, even at this age, a large heart, left ventricular strain, or increasing symptoms, indicate the need for it before it is too late. A surgeon of special experience should be chosen, but this problem of dealing with middle-aged patients should not arise in the future.

e. Atrial Septal Defect

The classical picture is a patient with a large heart, a tapping apex beat, a small pulse, and less disability than would be expected for the size of the heart. Generally the systolic murmur and thrill are not very striking: the pulmonary second sound is widely split but not very loud unless there is also pulmonary hypertension. In the electrocardiogram there is generally partial right bundle branch block, best shown in lead V_1, and there may be right ventricular preponderance and a long P-R interval. It is the only congenital condition in which auricular fibrillation is common, but this does not occur till late. The most characteristic feature is shown on radioscopy, the right side of the heart being large and the left side and the aorta small, and the pulmonary arteries and their branches being large and pulsatile. The shunt is generally large—between 50% and 70% of the inflow into the left atrium—and this happens with very little difference between the pressures in the two atria.

The outlook is good for many years in spite of the large shunt, but between 30 and 50 years of age many patients show increasing disability, sometimes with a rising pulmonary arterial pressure, and ultimately develop congestive failure. There is often some peripheral or pulmonary cyanosis but rarely complete reversal of the left-to-right shunt. Changes, however, come slowly, and young patients, even those with very large hearts, often do well with no further loss of ground for many years.

The poor ultimate outlook means that an operation as safe and successful as that for patent ductus would be advised widely, but at present the risk of operation for atrial septal defect is high. It should, however, be considered in patients who are losing ground and developing a rising pulmonary pressure before this has gone too far, and before the shunt becomes reversed.

f. Ventricular Septal Defect

The small ventricular septal defect, well known as the maladie de Roger, is not as common as has been thought, and most defects are larger and produce moderate or large shunts with pleonaemic lungs. The pressure in the right ventricle and pulmonary artery is more likely to rise than with other left-to-right shunts, and often in the teens or twenties the rise is enough to reverse the shunt, and the clinical picture is then indistinguishable from Eisenmenger's complex. There is a good review of ventricular septal defect by Wood, Magidson & Wilson (1954).

Closing a ventricular septal defect is not an easy or a safe operation at present: if it were, many of these patients would benefit from surgery. The present difficulties are technical, and it is hoped that they will be overcome in the future. The defect is nearly always high in the membranous part of the ventricular septum, and this produces surgical difficulties but they do not seem to be insuperable. At the moment, the main problem is the provision of safe methods of open surgery for the heart, and these are discussed elsewhere.[3]

It seems premature to decide the type of patients suitable for surgical treatment, but those with increasing symptoms and progressive enlargement of the heart, where the shunt seems likely to be reversed in the near future, would seem suitable.

[3] See papers by Brock (p. 189), Delorme (p. 221) and Ross (p. 226) in this number of the Bulletin.—Ed.

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INDIRECT OPERATIONS IN CYANOTIC HEART DISEASE

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1 Clinical management and anaesthesia
2 Routine operative practice
3 Blalock's (systemic-pulmonary) anastomosis
4 Potts' (aorto-pulmonary) anastomosis
5 Special problems
6 Post-operative management
7 Results

Most cases of cyanotic heart disease presenting for surgical treatment are variants of Fallot's tetralogy. Despite the theoretical and technical attractions of direct procedures such as pulmonary valvotomy with or without closure of the septal defects—now that hypothermia[1] is available for their correction—indirect procedures are essential for tricuspid atresia with rudimentary right ventricle and are still preferable for many cases, such as those in which there is any appreciable degree of pulmonary artery hypoplasia.[2] These indirect procedures are still preferred by a majority of surgeons—especially as the facilities required are simpler. Whilst " direct " operations may offer the greatest possibilities of restoration to normal and in themselves may actually be performed more quickly, they may be associated often with greater degrees of upset. Further recent developments and improvements of these methods require comparatively elaborate facilities, such as hypothermia and temporary arrest of the circulation. Whilst the future may well be with such operations, at present many surgeons—as has been said—prefer indirect procedures because of their relative simplicity, because they impose relatively little immediate strain on the circulation and because the immediate clinical results are almost invariably good.

Disadvantages of indirect operations are that, entailing somewhat tedious anastomoses, they are more time consuming and are liable occasionally to thrombotic occlusion. Special care must be taken that too big a shunt is not established, lest congestion of the pulmonary circulation with crippling or even fatal results follow. Also, only gradual resumption of activity must be allowed lest similar circulatory strain occurs.

1. Clinical Management and Anaesthesia

Patients requiring operative treatment for congenital cyanotic heart disease present many problems for the anaesthetist, and I am indebted to our Senior Anaesthetist, Dr Joan Millar, for the following observations.

Many are intensely cyanosed, dyspnoeic on the slightest effort, have little exercise tolerance and are prone to respiratory infections. Older patients may be very anxious for operative treatment and are helpful and co-operative. Frequently, however, the children are mentally backward, spoilt and difficult to deal with. At first sight they appear unpromising subjects for anaesthesia and operation; but in studying their medical histories, it is often found that they have survived quite serious childish illnesses and may have had tonsillectomy performed under general anaesthesia without serious consequences. Known respiratory infections should be controlled. Breathing exercises are taught and the patient accustomed to an oxygen tent, etc. We have not, however, found prolonged preparatory nursing in oxygen tents helpful. Adequate fluid intake is essential. Pre-medication is well tolerated and should be adequate for a smooth induction. We use Omnopon and scopolamine, in adequate dosage. A smooth induction is absolutely necessary. It is often easier to induce anaesthesia in a small child with *cyclo*propane or nitrous oxide and oxygen, but if a vein is available a small dose of sodium thiopentone is given. In children we use the largest plain Magill tube that will pass through the larynx. There is practically always an appreciable improvement in the patient's colour at this stage, possibly due to complete relaxation and reduced oxygen needs. Anaesthesia is maintained at a light level with nitrous oxide and oxygen, and a curarizing agent is used. It is necessary to provide as quiet an operational field as possible for the actual arterial anastomosis and, whilst the breathing is controlled by curarization, it must be maintained by rhythmical manual compression of the rebreathing bag. These delicate anastomoses may take some considerable time to perform, and it is necessary to ventilate as much lung tissue as possible without the lung obtruding too much into the operation field; thus, at suitable stages of the operation, the anaesthetist must re-inflate gently any collapsed areas of the lung. The surgeon and his assistants must avoid excessive or unnecessary compression or dislocation of either the lung or the heart, or traction on these. A slow intravenous infusion is maintained throughout the operation, and blood is kept available to compensate for haemorrhage. Many of these patients tolerate operation very well but a few will give grave anxiety throughout. One should not despair of such cases because recovery may follow almost desperate situations. Adrenaline and myocardial stimulants and electric defibrillatory apparatus must always be available for immediate use. It is our impression that cardiac irregularities due to manipulation of the heart have been minimized by the use of intravenous procaine. On completion of the anastomosis, the lung is gently re-inflated and any mucus removed by endobronchial suction. Oxygen, and small doses of sedatives when necessary, must be given post-operatively. Post-operative pulmonary complications are not infrequent, especially in children with a history of previous respiratory infections; usually, however, they are not unduly alarming.

Whilst hypothermia may be invaluable—and indeed essential—for some " direct " procedures, its role in connexion with indirect operations is at present hard to define. Generally speaking, increasing experience—on the part of both anaesthetist and surgeon—suggests that, with care and patience, comparatively desperate cases may be salvaged without its aid. It does seem, however, that hypothermia may have significant value where operation is imperative in severely cyanosed small infants.

[1] See papers by Delorme (p. 221) and Ross (p. 226) in this number of the Bulletin.—Ed.
[2] Cf. Brock, p. 189 of this number of the Bulletin.—Ed.

2. Routine Operative Practice

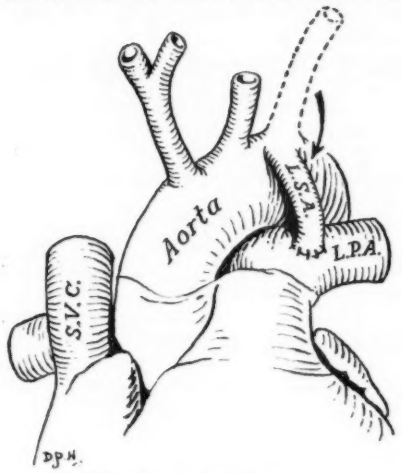
At operation our current practice is to explore these cases, after thorough catheterization and angiocardiographic investigations, through the left side of the chest, opening the pericardium and assessing the structural problem involved. If need be, direct-pressure recordings are taken by means of a cannula passed through the wall of the right ventricle along its outflow tract and into the pulmonary artery. Unless there is a definitely palpable stricture of the pulmonary valve and a normal-sized or dilated pulmonary artery with a definite pressure gradient uncomplicated by an " infundibular " stenosis, direct operation—it is felt at present—should not be further considered. Rather, the establishment of a shunt is proceeded with.

The desirability of establishing a shunt between the pulmonary and systemic circulations having been decided, a quick glance will usually suffice to decide whether the aorta itself lies close enough to the pulmonary artery or whether one of its branches—preferably the subclavian—is long enough to reach the pulmonary artery. The pulmonary artery is exposed by dissection in the mediastinum. On the left side it lies below the arch of the aorta above the bronchus and behind the upper pulmonary vein. On the right side the upper division of the artery is found between the bronchus and the superior vena cava, being slightly overlapped below by the upper part of the upper pulmonary vein. Compensatory vessels deep to the mediastinal pleura are sometimes varicocele-like in their extent and must be divided between ligatures one by one. This may be a tedious procedure but must be done with meticulous care so as to avoid troublesome—or even severe—haemorrhage. After mobilization the pulmonary artery should be gently occluded for four or five minutes as a test to gauge the tolerance of the circulatory system. A prolonged period of occlusion being necessary during completion of the anastomosis, this test is important because the pulmonary artery may be inadequate or even absent; if so, the test speedily causes deterioration of the patient's condition—a deterioration corrected by removal of the clamp. In such circumstances, unless a clamp permitting only partial occlusion of the pulmonary artery is available, the operation must be abandoned. The time occupied by this testing should be used for the dissection required to mobilize the aorta, or its branch chosen for the anastomosis.

3. Blalock's (Systemic-Pulmonary) Anastomosis

The subclavian artery that arises normally on the left side, directly from the aorta, is often convenient for this anastomosis (fig. 1), if the aortic arch is not so highly situated as to make the subclavian artery too distant from the pulmonary artery. It must be possible to obtain a length of artery long enough to prevent kinking at its origin from the aorta when it is turned over for the anastomosis. If the length of subclavian artery permits, it is desirable—to avoid disturbance of its collateral circulation—to divide it below the level at which it gives off its internal mammary, vertebral and neighbouring branches. On the other hand, section across the origins of these vessels is sometimes helpful by giving a wider mouth for the anastomosis. When the operation is done on the right side—as Blalock recommended—the subclavian branch of the innominate artery is similarly mobilized and brought down into proximity with the upper division of the right pulmonary artery (fig. 2). Bleeding from the subclavian artery is controlled

FIG. 1. BLALOCK'S ANASTOMOSIS OF LEFT SUBCLAVIAN ARTERY TO LEFT PULMONARY ARTERY

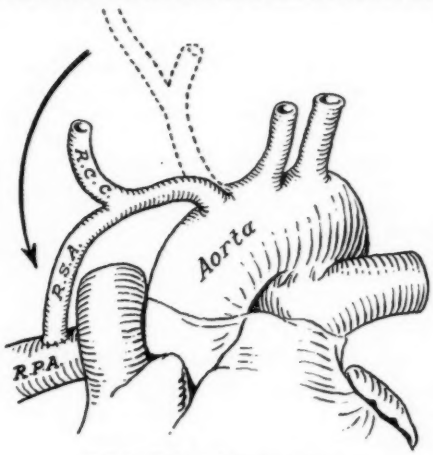


S.V.C.: superior vena cava
L.S.A.: left subclavian artery
L.P.A.: left pulmonary artery

by a small " bulldog " clamp applied to the vessel close to its origin before it is divided. The pulsations of the vessel sometimes tend to make this clamp creep towards the end of the vessel; this may be prevented by tying ligatures round the jaws of the clamp.

When the vessels are ready for anastomosis, a Derra clamp is applied to the pulmonary artery which is gently steered between the blades by traction of a silk ligature previously placed around the vessel. An incision of appropriate size is made in the wall of the pulmonary artery. The vessels are

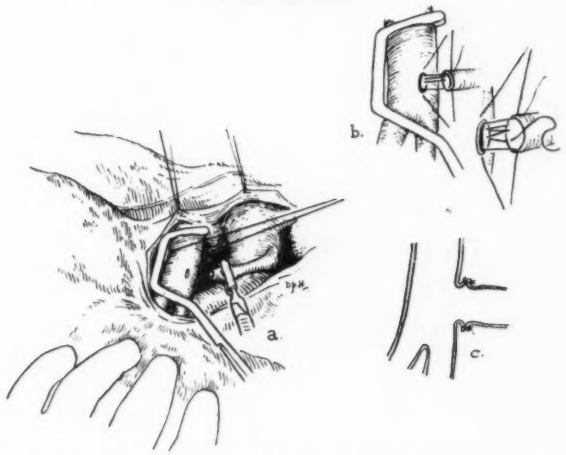
FIG. 2. BLALOCK'S ANASTOMOSIS OF RIGHT SUBCLAVIAN ARTERY TO RIGHT PULMONARY ARTERY



R.P.A.: right pulmonary artery
R.S.A.: right subclavian artery
R.C.C.: right common carotid

approximated by a Blalock suture, a simple running everting suture or by interrupted everting sutures—according to convenience—so as to give a flange-type junction. (See fig. 3.) Arterial silk (0000–00000) on atraumatic non-cutting needles is recommended for suturing. The distant side of the suture line is completed first, the bites of the sutures are placed 1–2 mm. apart, and all knots must be tied externally. It is useful to retain the first suture or the starting end of the suture as a marker and for traction; when the distant side is completed, the nearer edges are similarly approximated. Intermittently throughout the performance of the anastomosis, the operation field should be irrigated freely and gently with cold normal saline solution. This practice is advisable with all vascular anastomoses. When completed, the traction ligatures around the pulmonary artery should be held gently to ease strain on the suture line, and the Derra clamp opened. Bleeding often occurs from an occasional suture puncture, but usually ceases quickly when this is irrigated with cold saline or a gelatin sponge is applied. More serious bleeding may require re-application of the clamp and the insertion of additional sutures, but this should be avoided if possible as more bleeding may occur from these punctures. The clamp on the subclavian artery is then removed and, all being well, the anastomosis is now complete. The traction ligature around the pulmonary artery is removed. A thrill felt along the subclavian into the pulmonary artery indicates a functioning anastomosis. Kinking of the subclavian artery at its origin from the aorta may need removal of some adventitia, mediastinal fascia, etc., in the vicinity.

FIG. 3. BLALOCK'S ANASTOMOSIS: DETAILS OF PROCEDURE



a: pulmonary artery mobilized and pulled by silk traction ligature into jaws of Derra clamp. Subclavian artery—controlled by "bulldog" clamp—mobilized and ready for suturing to margins of small incision in pulmonary artery

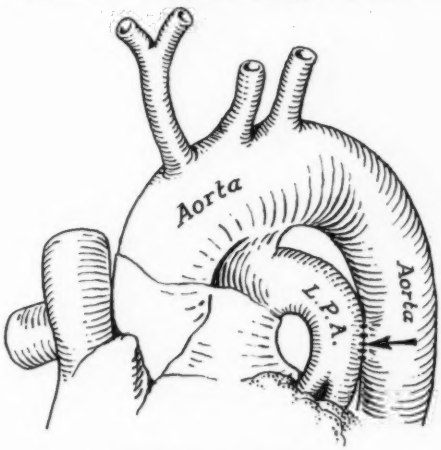
b: details of suturing to secure eversion—apposition of endothelium. Interrupted everting sutures are often used as an alternative method

c: diagrammatic representation of suture line

4. Potts' (Aorto-Pulmonary) Anastomosis

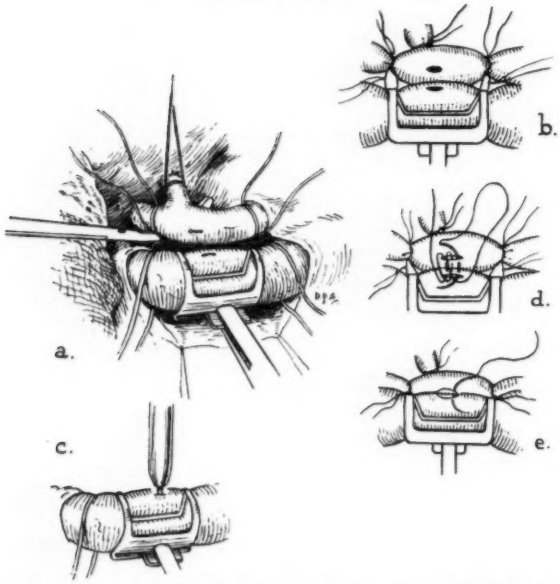
This operation we find preferable to that of Blalock, but it is practicable only when the aorta descends in its normal left-sided position (fig. 4). A length of the upper part of the descending aorta which will lie conveniently against the length of the left pulmonary artery already prepared is selected. It is mobilized by a division of two or three pairs of its intercostal and bronchial branches. This is much easier if an incision is made right through the mediastinal pleura overlying the aorta and the fascial sheath in which it lies. If the right plane is found in this way, we find there is much less trouble with bleeding from small (or even large) compensatory vessels. A Potts clamp of the appropriate size is then applied to this portion of the aorta, so as to present a lip to the vessel from which the blood flow is excluded, at the same time leaving a channel along the rest of the aortic lumen to maintain the circulation of the body below this level. When this latter channel is adequate, a definite thrill is felt in the aorta below the clamp. The presence of this thrill must be confirmed, lest irreparable damage to the spinal cord ensues. Naturally, the clamp should be applied in such a way that the lip presented for anastomosis lies towards the pulmonary artery. (See fig. 5.) Controlling silk ligatures are passed round the trunk of the left pulmonary artery high up, round its apical segmental branch and round the continuation of the trunk below this. The upper and lower of these controlling ligatures—which have been passed as slip knots round the artery—are then tied tightly to the blade of the Potts clamp. Gentle traction on the ligature around the apical branch keeps the site of the anastomosis in the pulmonary artery displayed adequately. An incision not exceeding 4 mm. in length is then made into the adjacent wall of both vessels. We have found Beck's disk punch of great help at this stage, the incision in the aorta being converted into a round hole which makes a completion of the anastomosis easier. A continuous arterial suture (0000–00000 braided silk) is used. Starting above, the deeper aspects of the vessels are brought together first by an over-and-over suture. After suturing the deeper side of the anastomosis, this is completed either with the same suture or a new one, as may prove convenient. The ligatures on the apical branch, that tying the lower trunk to the clamp and then that tying the

FIG. 4. POTTS' ANASTOMOSIS OF DESCENDING AORTA TO LEFT PULMONARY ARTERY



L.P.A.: left pulmonary artery

FIG. 5. POTTS' ANASTOMOSIS: DETAILS OF PROCEDURE



a: left pulmonary artery has been mobilized. Controlling ligatures have been passed round its trunk, on apical segmental branch and continuation of trunk below this. Potts clamp applied to aorta presents a desanguinated lip for anastomosis, whilst permitting blood to flow along rest of lumen. The short black lines on adjacent portions of the two vessels indicate site of intended anastomosis

b: upper and lower controlling ligatures around pulmonary artery trunk have been tightly tied to Potts clamp. Ligature around apical branch has been tightened and by traction on it serves to display pulmonary artery during anastomosis. An incision, not exceeding 4 mm. in length, has been made into adjacent surfaces of both vessels

c: Beck disk punch has been inserted through incision in aortic wall. The resulting round hole greatly facilitates anastomosis

d, e: suture tied externally approximates the deeper margins of anastomosis by an over-and-over stitch. Suture continued similarly completes anastomosis

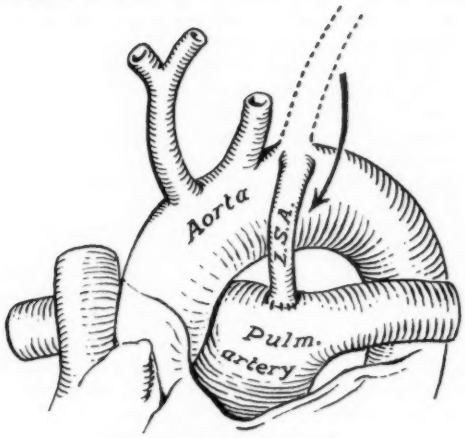
trunk above to the clamp, are released in that order. The Potts clamp is then released. If the anastomosis is satisfactory, a marked thrill becomes palpable in the pulmonary artery. When it is clear that all bleeding has ceased, the ligatures around the pulmonary artery—already slackened—are removed.

These anastomoses introduced by Blalock and Potts suffice for most cases. Circumstances are encountered occasionally, however, in which neither is practicable.

5. Special Problems

On occasion, whilst operating in the left side of the chest where the left pulmonary artery has been extremely small, I have found it possible to anastomose the subclavian artery to the main trunk within the pericardium (fig. 6). Then a small Potts toothed " bulldog " clamp—as used by Beck on the coronary sinus—has been helpful in isolating a small lip of the pulmonary artery for anastomosis with the subclavian artery

FIG. 6. ANASTOMOSIS OF LEFT SUBCLAVIAN ARTERY TO MAIN PULMONARY ARTERY WITHIN THE PERICARDIUM



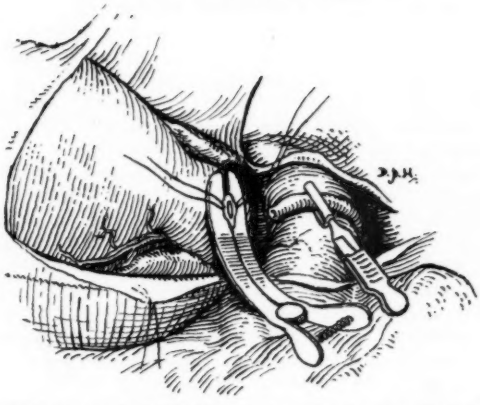
L.S.A.: left subclavian artery

without interrupting materially the flow along the former vessel (fig. 7). If the subclavian artery is too short for use in this way, it would be quite feasible to cut it off at its origin from the upper margin of the aorta and transplant it to a suitable point on the lower surface.

Sometimes—again during operation on the left side of the chest—the arch of the aorta may be situated too high to enable the subclavian artery to be mobilized sufficiently to reach the pulmonary artery, and the latter also may not be conveniently placed for a Potts anastomosis; then a free graft —as shown diagrammatically in fig. 8—may be useful. Whilst such a graft may be obtained from a " bank ", it is preferable to use one of the patient's own vessels if possible. Such a graft may be arterial or venous.

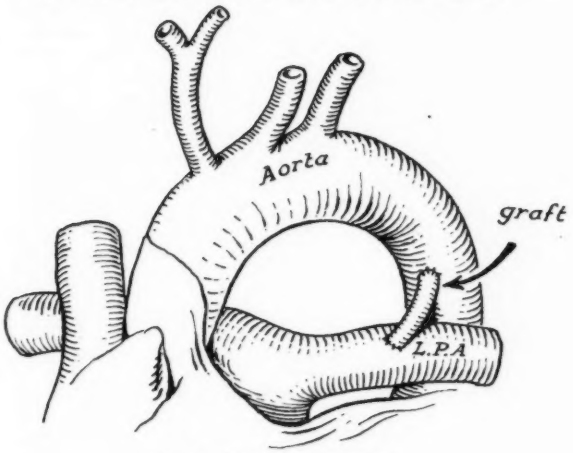
Figure 9 depicts a method which has given a particularly

FIG. 7. METHOD OF COMPLETING THE ANASTOMOSIS INDICATED IN FIG. 6



A small Potts toothed " bulldog " clamp has been applied to wall of pulmonary artery so as to present a small lip for anastomosis with subclavian artery, which has been mobilized and divided ready for suturing

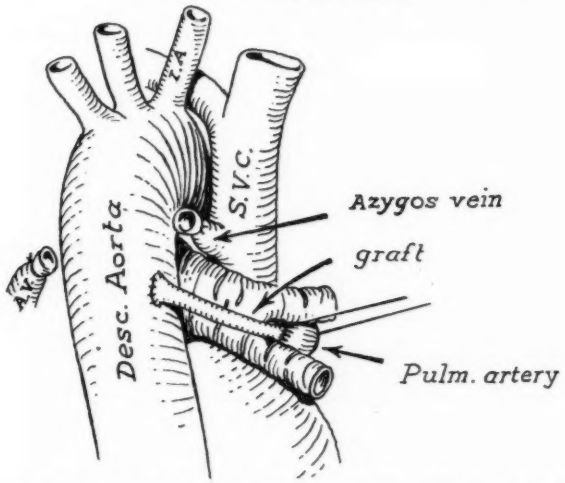
FIG. 8. DIAGRAMMATIC REPRESENTATION OF A FREE GRAFT (VENOUS OR ARTERIAL) BETWEEN LEFT PULMONARY ARTERY AND AORTIC ARCH



L.P.A.: left pulmonary artery

successful result in a difficult case where a previous pulmonary valvotomy and a subsequent left-sided pleurodesis had each in turn been disappointing. The patient was a child with a very high dextra-position of the aortic arch, with the aorta descending on the right side. At a third operation it did not appear feasible to anastomose the aorta or any of its main branches to the pulmonary artery. Indeed the pulmonary artery was situated unusually low down, deep in the front o the hilus. Accordingly, after it was mobilized between the upper and lower pulmonary veins, traction ligatures were passed round it and used to pull it through a space made in the angle posteriorly between the eparterial and lower stem bronchi. By manipulating these ligatures and applying a fine-toothed Beck aortic clamp, a suitable length of the artery was displayed for an anastomosis. The arch of the azygos vein was then used as a free graft.

FIG. 9. A METHOD OF ANASTOMOSIS USING A FREE GRAFT OF AZYGOS VEIN



The lower division of right pulmonary artery, having been mobilized and brought through angle between eparterial and lower stem bronchi by means of a temporary traction ligature, has been anastomosed to the descending (right-sided) aorta through a detached segment of azygos vein

S.V.C.: superior vena cava
A.V.: azygos vein
I.A.: innominate artery (left-sided)

Barrett, thinking that removal of the parietal pleura would encourage the formation of compensatory vessels between the pulmonary and systemic circulations, has suggested that this might be a helpful procedure where an anastomotic operation proves impossible or—perhaps because of the condition of the patient—unwise. He had observed that where an operation had been abandoned—say on the left side—for some such reason, with the intention of operating on the other side after an interval of some months, the second operation had subsequently seemed unnecessary because of improvement of the patient's colour and condition. There has been much controversy about this view but most clinics have had some such experience. It is probably true to say that in a small number of cases the mechanism can function beneficially.

6. Post-Operative Management

The pleura should be drained temporarily in all these cases. This enables early recognition of any material haemorrhage. It also largely limits compression of the lung by the almost unavoidable post-operative effusion. It is essential to have as full pulmonary ventilation and oxygenation as possible after operation. These patients should be nursed for the first post-operative days in oxygen tents.

Pyrexia has been a common post-operative—and often pre-operative—feature of our cases. This has often been so in the absence of other obvious evidence of infection, and it has been virtually unaffected by antibiotics. It is possible that a thrombotic element may be concerned, as in some cases where this has been accompanied by persistence or recurrence of the cyanosis, and by variations in the murmur of the fistula, improvement has followed the administration of heparin.

The importance of adequate rest after these operations cannot be too strongly emphasized. With infants the problem does not directly arise, but with older children there is a tendency for them to abuse their newly found mobility and considerably increase their physical activity because of their emancipation from a previously crippling anoxia. Some lead an increasingly active life with tremendous zest without harm ensuing, but others—inclined to too much activity—may develop increasing cardiac enlargement and signs of cardiac strain. If therefore there is any evidence of such progressive cardiac enlargement, the patient should be put to rest in bed, if necessary for many months.

7. Results

Technical skill and experience are of inestimable value in this work, but, these factors apart, the mortality of these " indirect " operations depends to a large extent on the type of clinical material presenting and accepted for such treatment. With an average group of cases—such as will be encountered in a regional unit—the mortality nowadays is probably assessed fairly at about 10%. If operation were

reserved for those who are reasonably fit, even though severely disabled—and such a combination is quite common—then this mortality would be much lowered. Contrariwise, the inclusion in the series of a high proportion of gravely ill infants would necessarily increase the mortality. It is equally certain that with expert management some of the most unpromising cases may be salvaged. Such cases must of course be accepted if they are to have any appreciable prospect of survival.

Those who survive these operations are usually greatly benefited and—although, to me, " direct " procedures have seemed more attractive—it has become apparent that we can rely more consistently on improvement after " indirect " operations. Objective evidence of benefit includes: improvement in colour, appearance and general behaviour, improvement in the blood picture (Table I), radiological improvement in the pulmonary vascular field (Plate I, figs. A, B), increased arterial oxygen saturation, and a quickening of physical growth and mental development as well as an obvious ability to undertake something approaching normal activity. These improvements are sustained.

Reference has been made to the importance of rest, with prevention and—if need be—treatment of pulmonary vascular congestion or of cardiac enlargement with failure, after these " shunt " operations. This strain on the circulation can be so severe as to cause death from pulmonary congestion within a few days of operation (Plate II, fig. C); such pulmonary congestion may fortunately be transient and a satisfactory result may be obtained, as in the case whose relevant radiographs are shown in Plate III (fig. D).

Cardiac enlargement and even congestive failure may become apparent within a few days of operation, but if recognition of this leads to delayed resumption of activity and limitation of it until an equilibrium is achieved, all may be well in the end. Cardiac enlargement after a " shunt " operation has seemed especially liable to occur where operation has been undertaken for tricuspid atresia. A Potts operation in one such infant (Plate III, fig. E) was followed by great cardiac enlargement and congestive failure. Prolonged bed rest for several months was followed by symptomatic recovery but the heart remained enlarged. Unfortunately death occurred 18 months later from whooping-cough. In another case of this type (Plate IV, fig. F), a young girl on whom the same type of operation was done developed cardiac enlargement but appeared otherwise well. Unfortunately she was allowed to go home and enjoy full activity; three weeks later she was readmitted with hemiplegia, further enlargement of the heart and congestive failure—hepatomegaly, oedema, etc. Gradual recovery followed complete bed rest for many months, and seven years later she remains in excellent condition.

TABLE I. BLOOD CHANGES FOLLOWING CREATION OF A PULMONARY-SYSTEMIC ARTERIAL SHUNT ON FIVE TYPICAL CASES OF FALLOT'S TETRALOGY

(A Potts operation was done in three of these cases and a Blalock operation in two. There is improvement in each instance.)

Age of patient (years)	Operation	Haemoglobin (%)*		Erythrocytes (millions)		Haematocrit reading (%)	
		Pre-operative	Post-operative	Pre-operative	Post-operative	Pre-operative	Post-operative
6	Blalock	153	135	7.28	6.89	65	65
7	Potts	143	120	7.80	6.20	86	56
8	Potts	128	94	6.4	4.8	67	48
12	Blalock	161	124	7.63	6.45	70	59
13	Potts	146	106	7.3	6.5	85	56

* Haldane scale.

ACKNOWLEDGEMENT

I am indebted to Mr D. P. Hammersley, King's College, University of Durham, for his skilful illustrations of the operations described.

* * *

INDIRECT OPERATIONS IN CYANOTIC HEART DISEASE

(FIGS. A—F)

G. A. Mason

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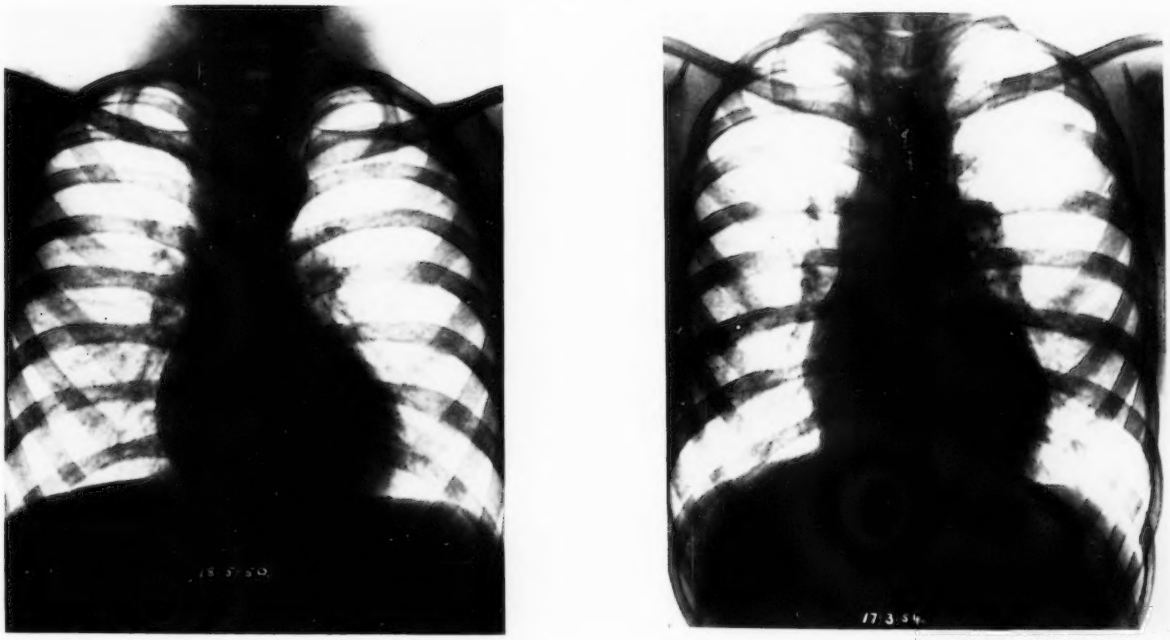
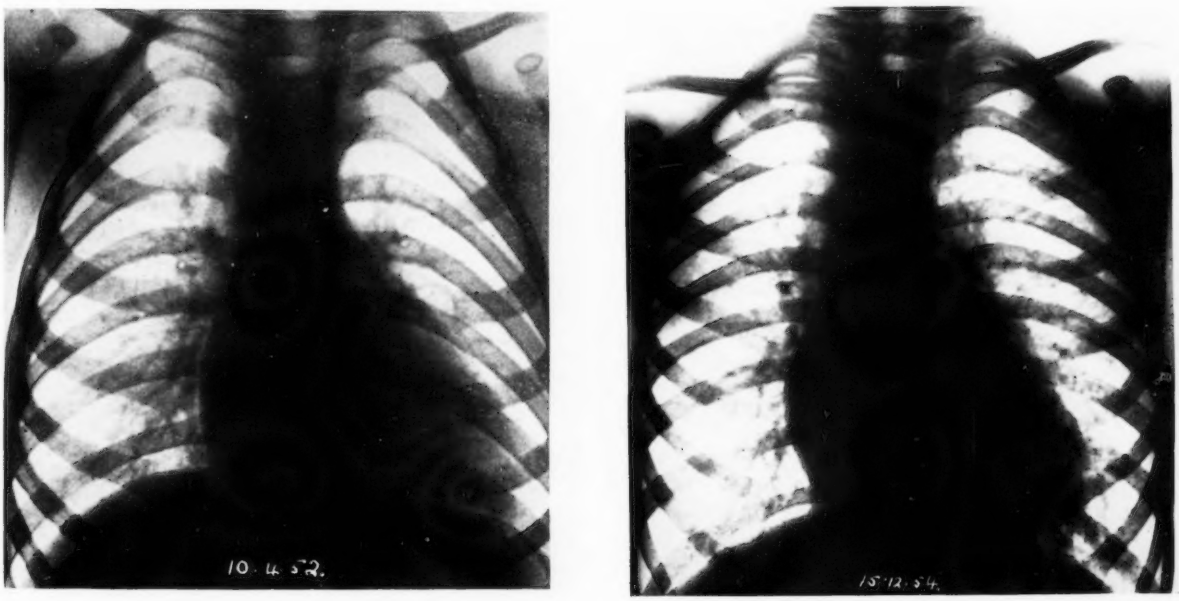


a b

FIG. A. Radiographs of young boy with Fallot's tetralogy treated by aorto-pulmonary anastomosis

a: pre-operative appearances

b: note increased distension of pulmonary arteries four years later



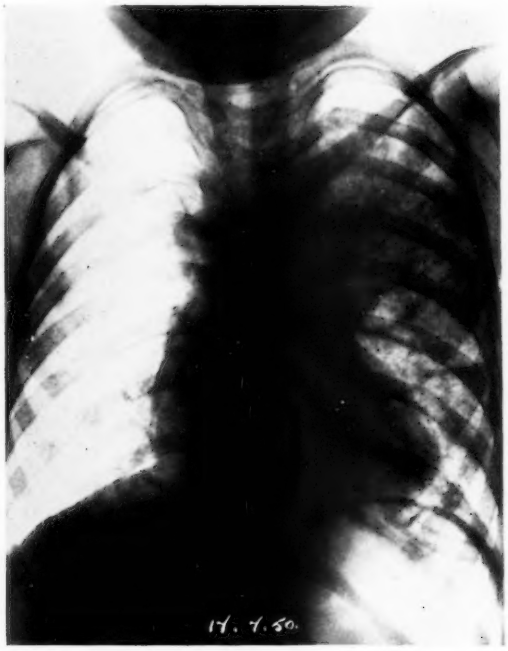
a b

FIG. B. Radiographs of young child with Fallot's tetralogy treated by subclavian-pulmonary artery anastomosis

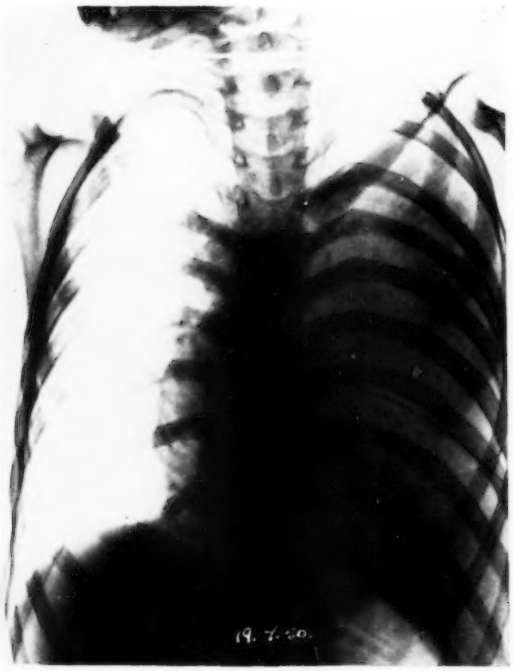
a: pre-operative appearances

b: showing sustained vascular improvement in pulmonary field two years later

a

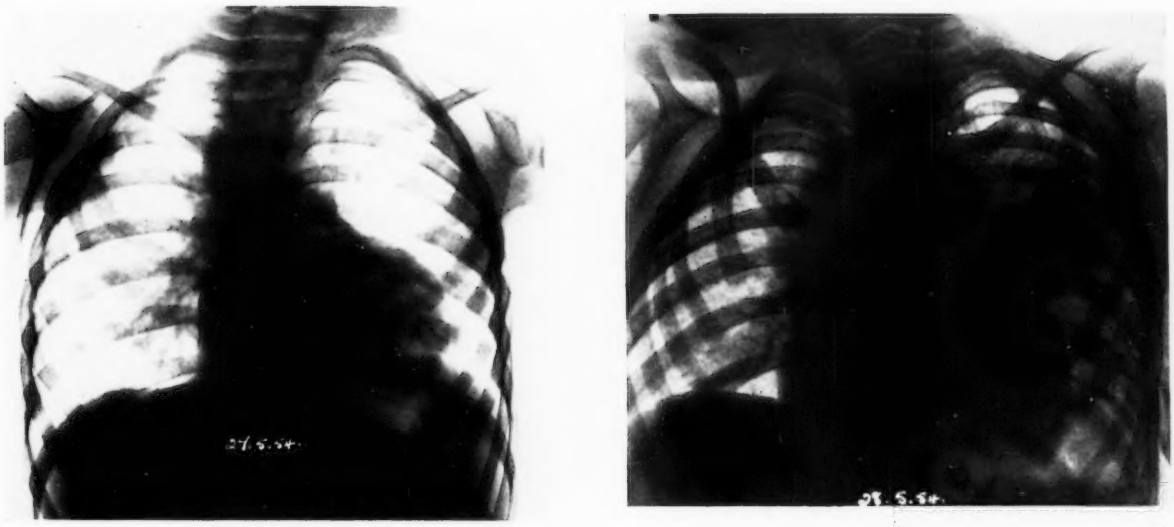


b



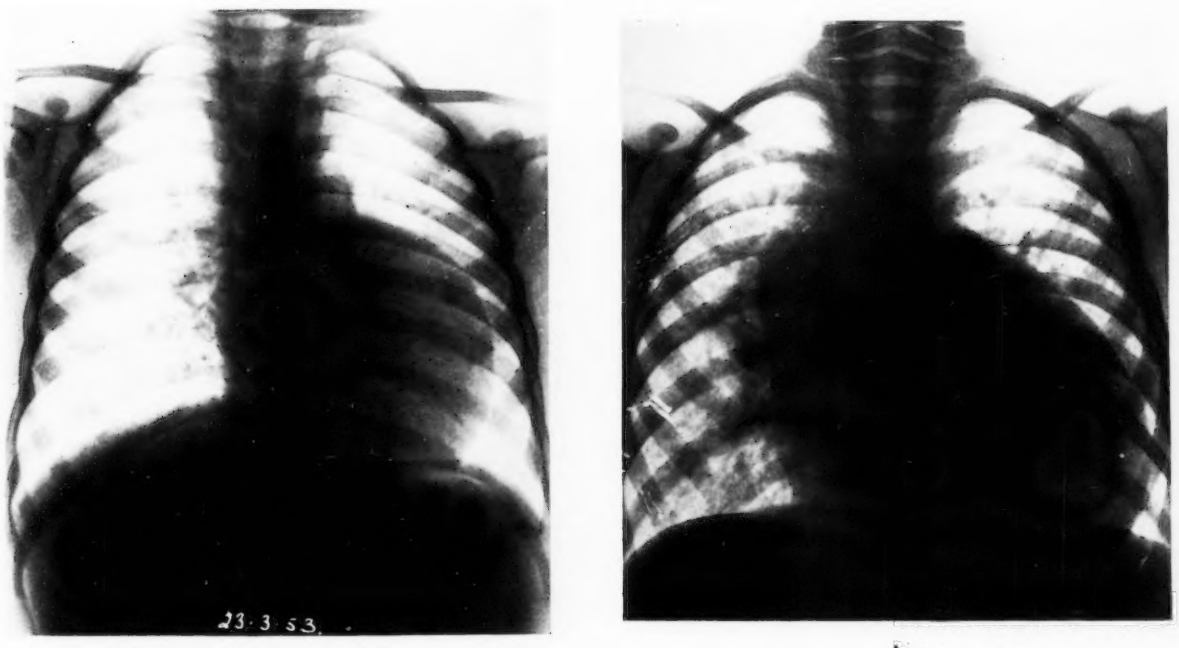
c

FIG. C. Radiographs of young child with Fallot's tetralogy treated by aorto-pulmonary artery anastomosis
(a) pre-operative appearances. After completion of anastomosis an unusually marked thrill was palpable in upper lobe branches of pulmonary artery, and there was a striking improvement in patient's colour. Later on same day child's general condition caused anxiety, and radiograph (b) showed some extravasation in, or consolidation of, upper lobe of left lung. The child's condition further deteriorating, it died three days later, the extravasation—confirmed at necropsy—spreading throughout the whole lung (c)



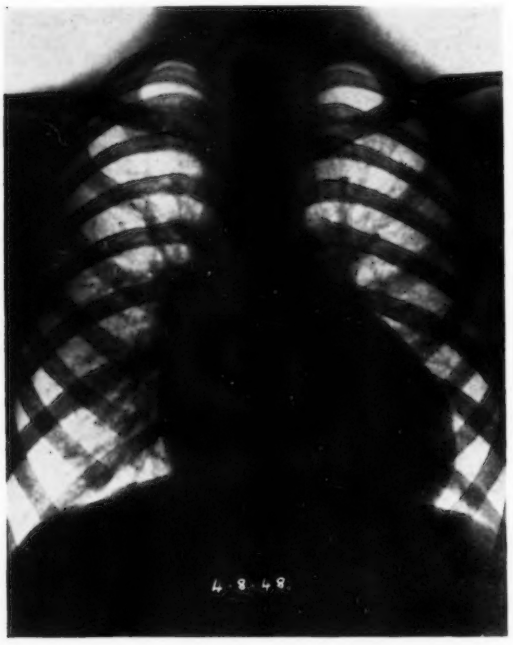
a b

FIG. D. A boy who had been operated upon for congenital oesophageal atresia shortly after birth—18 months previously—also had Fallot's tetralogy (a) for which a Blalock anastomosis was done. On the following day there was a marked pulmonary oedema (b) but spontaneous recovery was made and six months later the general condition was excellent and the radiological appearances of the lung fields satisfactory; treatment of the oesophageal stricture continues



a b

FIG. E. Radiographs showing (a) pre-operative appearances of a young infant with tricuspid atresia. Cardiac enlargement and congestive failure occurred after aorto-pulmonary artery anastomosis. Complete symptomatic recovery followed prolonged rest although the heart remained enlarged (b). Death occurred 18 months later from whooping-cough



a

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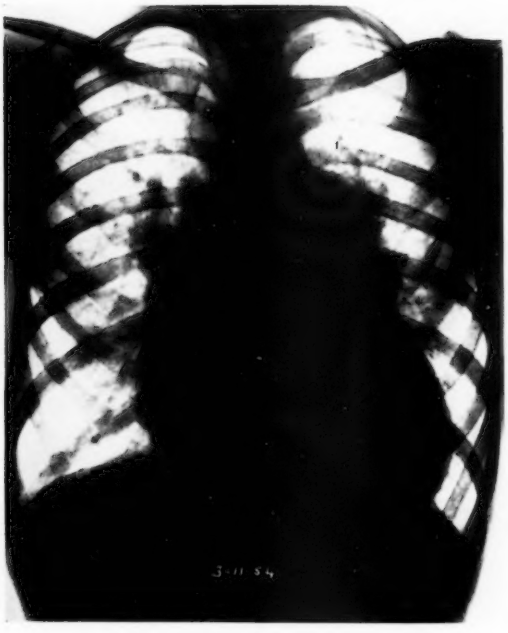

d

FIG. F. Radiographs showing (a) pre-operative appearances of a young girl with (presumed) tricuspid atresia. The heart became enlarged after aorto-pulmonary artery anastomosis (b). Congestive failure and hemiplegia occurred shortly afterwards, but the patient recovered after many months of complete bed rest. Although the heart remains large (c, d) the patient is in good health, of normal, rather high, coloration and leads the active life normal for a girl of her age, six years later

DIRECT OPERATIONS IN THE TREATMENT OF PULMONARY STENOSIS

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1 Pulmonary valvotomy
2 Fallot's tetralogy
  a Basis of direct operations
  b Anatomical basis
  c Results
  d Cyanosis
  e Secondary effects on heart and pulmonary circulation
References

In 1945 Blalock & Taussig published their epoch-making work on the treatment of certain cases of congenital cyanotic heart disease by means of a systemic-pulmonary anastomosis. This operation was based on their postulate that the patient's cyanosis and disability are due to deficient blood flow to the lungs. The correctness of this was amply proved by the excellent results of their operation in a high percentage of cases. Potts, Smith & Gibbons (1946) later introduced the modification of aorto-pulmonary anastomosis.

At that time the differential diagnosis of the various types of pulmonary stenosis was not so advanced as now, and in the first rush of cases submitted to operation were a number of examples of a condition quite unsuitable for this type of procedure. This condition is pulmonary valve stenosis with a normal aortic root. Sometimes it exists with both septa closed, in which case there is no cyanosis. Sometimes there is a communication between the two atria which causes a right-to-left shunt and cyanosis: the so-called "trilogy of Fallot." At the time of Blalock and Taussig's work pulmonary valve stenosis was thought to be so rare as to be almost a curiosity. We now know it occurs as commonly as persistent ductus arteriosus, as shown by Campbell (1953) in a study of 1,130 cases of congenital heart disease and by Wood, Magidson & Wilson (1954). For an account of the clinical and other features the reader is referred to articles by Campbell, Deuchar & Brock (1954), Allanby & Campbell (1949) and Abrahams & Wood (1951).

The real trouble in pulmonary valve stenosis is obstruction of the right ventricle; the actual cyanosis is due to a secondary rise in pressure in the right atrium. If a Blalock or a Potts operation is done, the obstruction to the right ventricle is not relieved, a new strain is imposed upon the heart by the extra work the left ventricle has to do, and the increased blood flow to the lungs aggravates the hold-up in the right heart. Right-sided failure sooner or later supervenes.

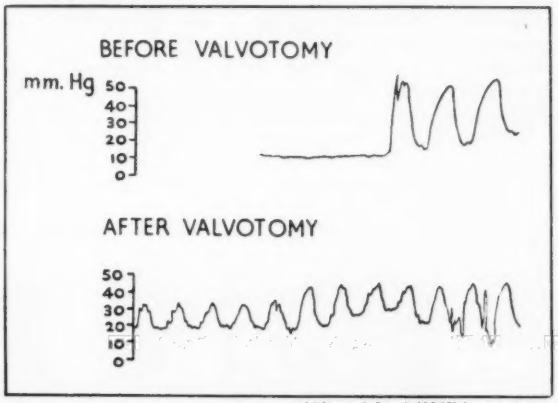
1. Pulmonary Valvotomy

It is now universally accepted that the anastomotic operations have no place in the treatment of pulmonary valve stenosis and that the only proper treatment is direct relief of the obstruction by pulmonary valvotomy. This operation was first done by Brock (1948) and by Sellors (1948). Brock & Campbell (1950) and Campbell & Brock (1955) presented and emphasized fully the correct basis of treatment by valvotomy as opposed to short-circuiting the obstruction.

A large number of pulmonary valvotomies have now been done and we have recently presented our experiences in a critical analysis of 58 cases (Campbell & Brock, 1955). The reader should consult this article for much detail that is lacking in the following account of 94 cases, which is the largest series so far presented.

Full operative details are given in an article by Milstein & Brock (1955), and it must suffice to state here that the method used in all these 94 cases was the transventricular, i.e., after exposure of the heart an incision is made in the wall of the infundibulum of the right ventricle, a cutting valvotome is inserted and then dilating instruments to open the valve widely. Pressure readings are taken before and after valvotomy to assess its efficacy (fig. 1).

FIG. 1. DIRECT ELECTROMANOMETRIC TRACINGS MADE AT OPERATION, BEFORE AND AFTER PULMONARY VALVOTOMY FOR PULMONARY STENOSIS



Milstein & Brock (1955) by permission of Guy's Hospital Reports

Note almost complete obliteration of the gradient across the valve.

In the first cases the mortality was high, partly owing to inexperience with the operation, but more because advanced, virtually hopeless, cases were selected. The mortality is now low, as can be seen from Table I.

TABLE I. PULMONARY VALVE STENOSIS

Total number of cases.	94
Number of deaths*	13
Deaths (percentage of total)† . . .	14

* 7 deaths occurred in the last 87 cases (8%).
† Deaths occurred at ages 37, 36, 29, 27, 25, 22, 22, 20, 19, 15, 14, 9 and 9 years.

It should be noted that most of the deaths occurred in patients over 20 years of age; in these the disease was much advanced. The grave significance of cyanosis is shown by the

mortality in the cyanotic and acyanotic groups. Of 60 patients with cyanotic disease, 12 died; of 34 patients with acyanotic disease, one died.

The results of the operation are good, as shown by the following broad survey:

Number of survivors . .	81
Very good (or good) results .	73 (90%)
Improved	8 (10%)

Campbell (Campbell & Brock, 1955) presents a more detailed analysis of the results in 58 of these cases.

In many there is a striking diminution in the size of the heart (Plate V, fig. A) and also great improvement in the electrocardiogram. Figure 2 shows the change from severe right ventricular hypertrophy and strain to only moderate right hypertrophy.

Catheter studies made before and after operation in 24 cases show that pulmonary valvotomy is very effective in reducing the systolic pressure in the right ventricle; in all the 24 patients it was reduced to less than half its previous level. The pressure gradient fell from an average of 122 mm. to 42 mm. Of nine cyanotic cases two showed a fall to normal and in most the systolic pressure fell to between 45 mm. and 60 mm. Equally important, the right-to-left shunt was reduced and generally abolished. On the average the oxygen saturation in the systemic arterial blood rose from 78% to 94%. The pulmonary flow increased from 2.5 l./min. to 4.2 l./min. Of the 15 acyanotic patients none showed a normal right ventricular systolic pressure, but in most it was between 40 mm. and 60 mm.

In all but a few cases, therefore, some degree of pulmonary stenosis persists, but this is slight instead of severe and gives rise to few, if any, symptoms. The pressure in the right ventricle is usually well below the level at which, if met with in a case not operated on, operation would be advised.

Recently Swan, Zeavin & Blount (1953) and Blount, McCord, Mueller & Swan (1954) have advocated open transarterial valvotomy under hypothermia[1]; by arresting the circulation, the pulmonary valve can be exposed and divided under direct vision. In theory this is certainly preferable to any closed method, but it is as yet too early to show that this method gives consistently better results with a satisfactorily low mortality. In most of Swan and Blount's cases the pressure gradient across the valve was completely obliterated, but there was a disconcertingly high incidence of pulmonary regurgitation, a condition rarely seen in our series.

2. Fallot's Tetralogy

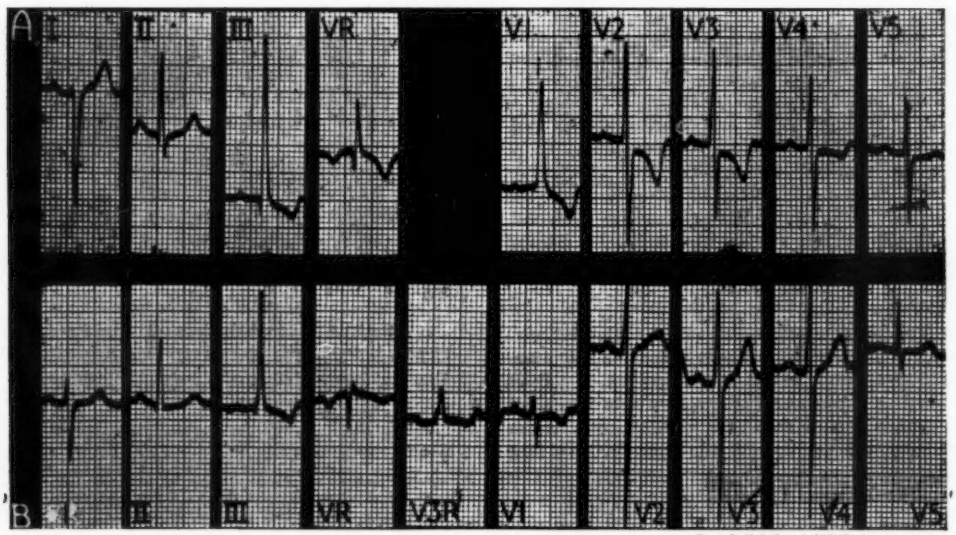
Opinion is divided about direct operations for the relief of pulmonary stenosis in Fallot's tetralogy. Although in theory many accept the arguments that the direct operations are better founded than the indirect procedures of Blalock and Potts, in practice the direct operations have not been extensively used through fear that they are technically more difficult, that the mortality is higher and the results not so good as with the anastomotic operations. It will be shown that this is not so; that the operations are just as safe and the results are just as good.

a. Basis of Direct Operations

The brilliant immediate results given by the indirect operations should not blind us to certain disadvantages. The heart

[1] See papers by Delorme (p. 221) and Ross (226) in this number of the Bulletin.—Ed.

FIG. 2. ELECTROCARDIOGRAPHIC CHANGES BEFORE AND AFTER PULMONARY VALVOTOMY



Campbell & Brock (1955) by permission of British Heart Journal

Note the great diminution of right ventricular hypertrophy and of right ventricular strain.

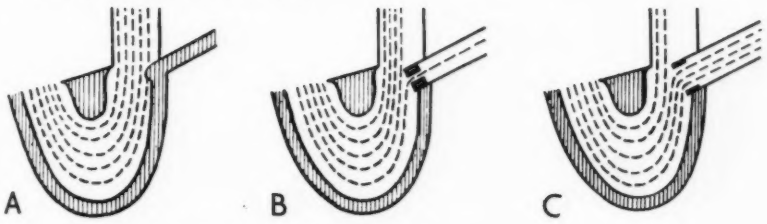
A: before operation

B: after operation

lesion in Fallot's tetralogy is initially very complicated; a systemic-pulmonary anastomosis adds yet another abnormality by making an artificial ductus arteriosus. Failure to relieve the obstructed right ventricular outflow not only prevents normal growth, but is also followed by steady increase in the severity of the pulmonary stenosis from organization of deposits of fibrin and platelets and from formation of verrucose vegetations. Ultimately the pulmonary stenosis becomes virtually complete, which means that life is supported by artificial bronchial arteries—conditions scarcely compatible with a long expectation of life.

The direct operations, which seek to relieve the pulmonary stenosis, lessen the obstruction to the right ventricle and increase the blood flow to the lungs by the natural route. At the same time there is a physiological lessening or correction of the right-to-left shunt (fig. 3). The increased passage of blood through the pulmonary outflow allows its natural growth with age. Thus, steps are made towards correction of the cardiac abnormalities.

FIG. 3. DIAGRAM TO ILLUSTRATE THE PHYSIOLOGICAL DIMINUTION OF THE RIGHT-TO-LEFT SHUNT PRODUCED BY DIRECT RELIEF OF A PULMONARY STENOSIS



A: showing complete shunting in pulmonary atresia

B: showing a big right-to-left shunt

C: showing that right-to-left shunt has been lessened by relief of the pulmonary stenosis

Objections have been raised to the direct operations, on several grounds. These may be detailed as: (i) they are anatomically unsound; (ii) they are dangerous; (iii) the results are poor; (iv) they may cause an Eisenmenger complex.

b. Anatomical Basis

Space does not permit a detailed presentation of the anatomical basis of the direct relief of pulmonary stenosis. It must suffice to say that most of the arguments brought against it are the result of observations made on dead hearts which are either in a state of firm contraction from rigor mortis or have been rendered even harder and more contracted by formalin fixation. The heart is a living, active, pulsating structure, and observations made in the post-mortem room cannot be compared favourably with those made on the heart during life at operation; the heart can then be seen and felt in function, its chambers can be opened and palpated, even inspected, and the pressure changes studied in its various parts. In this way it is possible to demonstrate that in a high proportion of cases the pulmonary stenosis is of a type that can be corrected by some direct operation such as valvotomy, infundibular resection, or a combination of the two. The best and most convincing argument for the correctness of the anatomical basis of the direct operations is the excellence of the results; these could not be achieved if the operations were ill founded.

c. Results

The statements that the mortality is high and the results poor can come only from ignorance and lack of experience, for they have no basis of reality. This is amply shown by Table II, which gives the mortality and results in 140 direct operations.

It will be seen that both the mortality and the results compare favourably with the two series of indirect operations. It is certain that the direct operations are no more dangerous and the clinical results are just as good.

Table III shows the comparative mortality of the different direct procedures.

TABLE II. FALLOT'S TETRALOGY: RESULTS IN 140 DIRECT OPERATIONS AND IN TWO SERIES OF INDIRECT OPERATIONS

	Direct		Indirect	
Result	Number of cases	Percentage of total	Campbell & Deuchar (1953) (165 cases) Percentage of total	Taussig & Bauersfeld (1953) (857 cases) Percentage of total
Very good or good	107	76	77.5	78
Improved	16	11.5	8.5	4
Not improved	1	0.7	5.5	3
Died	16	11.5	8.5	15

Table III completely refutes the statement that infundibular resection carries a high mortality; the figure of 13% mortality compares very favourably with the world figures for the anastomotic procedures.

Table IV gives the comparative results; it can be seen that infundibular resection carries no appreciable extra risk.

d. Cyanosis

After a Blalock or a Potts operation, cyanosis lessens; at rest the colour often appears quite normal, and after exercise some degree of cyanosis appears. The arterial oxygen saturation is rarely or never as high as 90%. After the direct operations the colour is usually quite normal even after exercise; the arterial oxygen saturation is often over 90% and not infrequently over 95%.

TABLE III. COMPARATIVE MORTALITY OF DIFFERENT DIRECT PROCEDURES

Operation	Total number of cases	Number of deaths	Deaths expressed as percentage of total
Valvotomy	50	6	12
Infundibular resection	62	8	13
Combined valvotomy and infundibular resection	28	2	7
Total	140	16	11.5

TABLE IV. FALLOT'S TETRALOGY: COMPARATIVE RESULTS OF INFUNDIBULAR RESECTION IN 140 CASES

Operation	Very good and good (%)	Improved (%)	Not improved (%)	Died (%)
Valvotomy (50 cases)	74	12	2	12
Infundibular resection (62 cases) .	80	7	—	13
Combined infundibular resection and valvotomy (28 cases) . . .	72	21	—	7

e. Secondary Effects on Heart and Pulmonary Circulation

A common criticism of the direct operations is that they will result in a gross elevation of the pulmonary arterial pressure and that a state similar to the Eisenmenger complex will result. In our experience this is not so, although it is possible that it could be caused by an excessive relief of the stenosis. In general, although the pulmonary blood flow is greatly increased, the high pressure in the right ventricle is not transmitted to the pulmonary arteries.

Studies by cardiac catheterization before and after operation in 16 cases have revealed the following facts. (i) The oxygen saturation rises from an average of 78% to an average of 91% (in eight cases it was more than 90%, and in four cases it was more than 95%, after operation). (ii) The average increase in pulmonary artery pressure is not great. (iii) The systemic flow remains the same. (iv) The pulmonary flow rises from an average of 3.1 l./min. to 4.9 l./min. If this is expressed as a percentage of the systemic flow, the pulmonary flow increases by about 50–100%. (v) In six cases, the operation has reversed the right-to-left shunt to a left-to-right one, as shown by the arterial oxygen saturation being over 95% and the pulmonary flow becoming greater than the systemic flow. One case will serve as an example: oxygen saturation rose from 86% to 95%; pulmonary flow increased from half to double the systemic flow; pulmonary artery pressure rose from 14/7 mm. Hg to 38/12 mm. Hg.

When the right-to-left shunt is overcorrected in this way certain untoward features are observed. It is inevitable that the heart should show some enlargement after a successful operation, because the right ventricle continues to beat against the peripheral resistance, and also the patient is more active than before. Ordinarily this enlargement is not much, but in some cases when the clinical result is very good and the cyanosis completely relieved, even on exercise, the enlargement may be rather more than seems desirable (Plate V, fig. B); this is also seen after the anastomotic operations.

In conformity with this the electrocardiogram may develop untoward features as the years pass. The right ventricular preponderance remains after operation, but in some cases evidence of right ventricular strain may develop after either the indirect or the direct operations, especially when the heart size has increased too much. As shown in fig. 4, T inversion appears from V_1 to V_4 or even V_5. This is in marked contrast to valvotomy for pulmonary stenosis with a normal aortic root, in which gross electrocardiographic changes present before the operation gradually revert to normal (fig. 2). However good the clinical result is, these electrocardiographic changes, coming on perhaps after several years and indicating a strain on the right ventricle, must arouse some disquiet.

Although the pulmonary stenosis may have been satisfactorily relieved, the heart is still not completely normal; the septal defect and the overriding aorta remain and these continue to impose a burden on the heart.

As at present practised neither the direct nor the indirect type of operation gives fully satisfactory results which allow us to promise a safe and satisfactory long-term prognosis to the patient or the parents, although in this respect the direct operations are superior, as the heart is rendered more nearly normal.

But surgery is not static: it is a living and developing force. If we accept the results of the indirect procedures and decide to practise them to the exclusion of all else, then we have come to a halt. The problem is finished; there can be no further development.

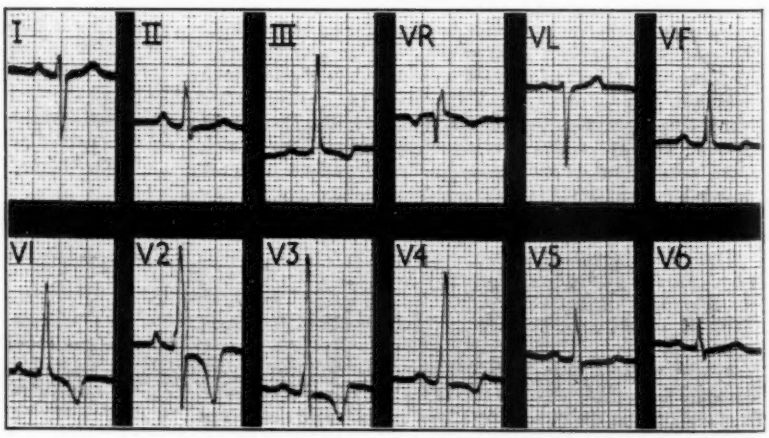
The direct procedures, however, are different; their perfection and further development is limited only by technical matters. At present we relieve the pulmonary stenosis by somewhat blind methods, not entirely satisfactorily. The ventricular septal defect and the overriding aorta remain as a threat to the long-term result; the doubtful prognosis pertaining to these replaces the more immediate danger of the pulmonary stenosis and the right-to-left shunt.

Surgery is developing all the time. Even now, with the extra facilities that the use of hypothermia[2] has given us, we can open the pulmonary outflow tract, see the stenosis directly

Continued at foot of page 193

[2] See papers by Delorme (p. 221) and Ross (p. 226) in this number of the Bulletin.—Ed.

FIG. 4. ELECTROCARDIOGRAPHIC CHANGES AFTER OPERATION FOR FALLOT'S TETRALOGY



Campbell, Deuchar & Brock (1954) by permission of British Medical Journal

Note the development of right ventricular strain.

DIRECT OPERATIONS IN THE TREATMENT OF PULMONARY STENOSIS

(FIGS. A, B)

Sir Russell Brock

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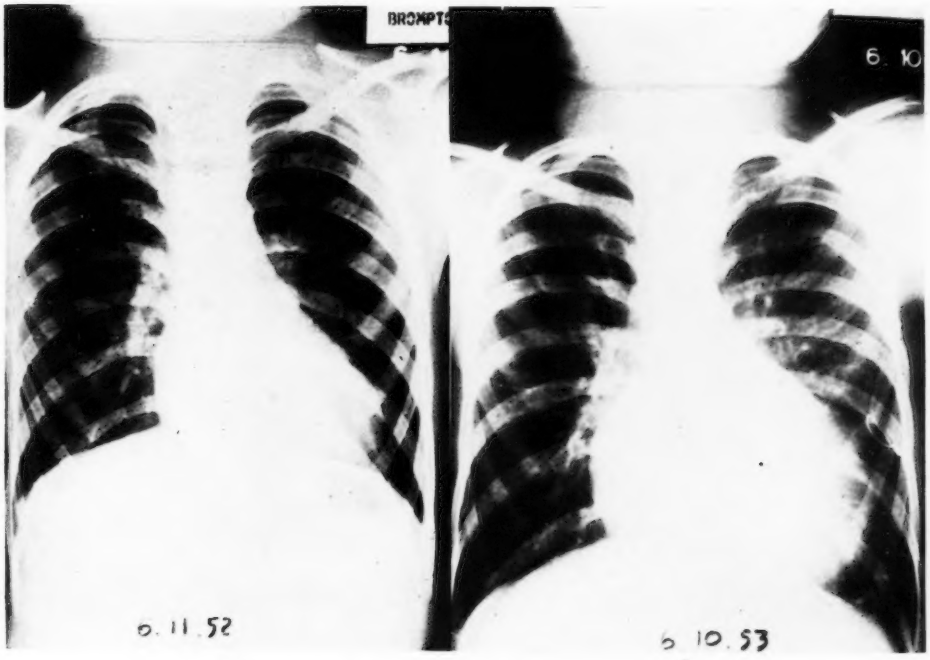
a b

FIG. A. Radiographs of heart (a) before and (b) after pulmonary valvotomy, showing diminution in size



a b

FIG. B. Radiographs of heart (a) before and (b) after operation for Fallot's tetralogy, showing excessive increase in heart size

PLATE VI (FIGS. A, B)

SURGERY OF SEPTAL DEFECTS

(FIG. A)

D. N. Ross

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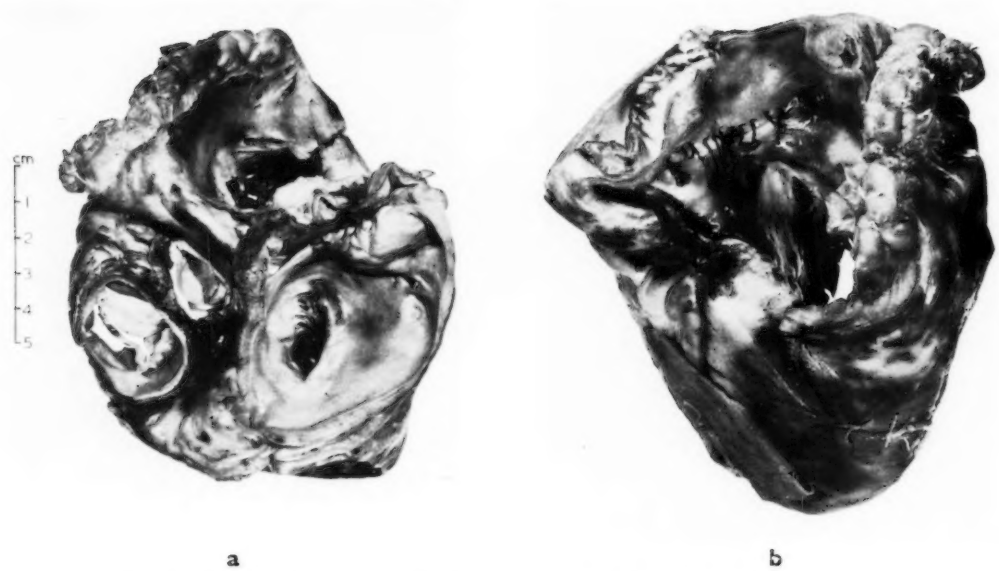

a b

FIG. A. Autopsy specimen of a repaired atrial septal defect with minor degree of coexisting mitral stenosis
a: mitral orifice b: closed defect viewed from right atrium

SURGICAL TREATMENT IN COARCTATION

(FIG. B)

O. S. Tubbs

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FIG. B. Chest radiograph of a girl aged 11 wi coarctation of the aorta which involved the l subclavian artery. Notching confined to the rig side is well seen in the 4th and 5th ribs, and, to slight extent, in the 6th and 7th ribs

SURGERY OF SEPTAL DEFECTS

The Present Position

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1 Defects of the atrial septum
2 Principles of treatment
3 Methods of closure
  a Closed techniques
  b Open methods
4 Defects of the ventricular septum
5 Aorto-pulmonary septal defects
References

The surgical correction of septal defects has received considerable impetus from the recent developments of methods for open cardiac surgery. At the same time many ingenious closed techniques have been developed experimentally and applied clinically in order to avoid the hazards of ventricular fibrillation and air embolism that are liable to complicate open operations on the septa.

Since atrial septal defects constitute the commonest congenital cardiac defect (Wood, Magidson & Wilson, 1954), it is not surprising that efforts have been directed towards their closure. Swan, Zeavin & Blount (1953) have offered convincing proof that hypothermia[1] appears to be the best available technique at present. Ventricular septal defects occurring as an isolated anomaly are relatively uncommon, but as part of the picture of Fallot's tetralogy their correction deserves urgent attention. The recently described technique of cross-circulation (Warden, Morley, Read & Lillehei, 1954)[2] is an important development in this field, but suffers from the problem of danger to the donor. Mechanical heart-lung machines have proved disappointing in the provision of a safe dry-heart technique, and this is probably related to the inadequacy of mechanical oxygenators in performing the multiple biological functions of lung tissue.

1. Defects of the Atrial Septum

The haemodynamic disturbance in this condition arises as a result of the left-to-right shunt of blood between the atria. If there is an additional anomalous pulmonary venous drainage, the amount of blood being pumped around the lesser circulation may be very many times that passing into the aorta. The coexistence of mitral stenosis and an atrial septal defect will again increase the volume of the left-to-right shunt (Plate VI, fig. A).

The commonest form of atrial septal defect lies posteriorly and represents a defect of the septum secundum, while the less common deficiency of the septum primum results in a defect low down and overriding the atrioventricular valves. The proximity of these valves increases the technical difficulties of the closure of the ostium primum defect and resembles in this respect the problems associated with the closure of high ventricular septal defects. The pre-operative recognition of these ostium primum defects is therefore of primary importance. Many of the clinical features of this condition arise as a result of incompetence of the adjacent mitral valve. We therefore expect to find the systolic murmur rather lower down in the region of the apex and again, because of the valvular incompetence, symptoms are likely to be manifest at an earlier age than in uncomplicated defects of the ostium primum.

[1] See Ross, p. 226 of this number of the Bulletin.—Ed.

[2] See also Andreasen, p. 233 of this number of the Bulletin.—Ed.

DIRECT OPERATIONS IN PULMONARY STENOSIS

Continued from page 192

and resect it quickly and simply. This we have done successfully on four occasions, and several other surgeons have done the same.

At the same time the interventricular septal defect is displayed and can be closed by direct suture. In some cases with gross dextro-position of the aorta and a large defect, correction may be impossible, but in others satisfactory closure will be achieved. We need not then be worried about too great relief of the pulmonary stenosis; in fact the pulmonary outflow tract can be made as large as possible. The pulmonary arteries and the right ventricle will be protected from the output of the left ventricle and from the systemic resistance, and the heart will be virtually normal. Thus we shall be able to offer with confidence a good, long-term prognosis to our patients.

This is not imagination. We have already twice closed a ventricular defect and excised the pulmonary stenosis in Fallot's tetralogy under the direct vision permitted by hypothermia; unfortunately both these patients died, but Lillehei and his colleagues have been completely successful in their cases treated by the help of cross-circulation (results reported at International Symposium on Cardiovascular Surgery, Detroit, but not yet published). It is only a matter of technical development before we are able to achieve this almost routinely and to offer our patients a complete cure, a state that no indirect operation can ever achieve.

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FIG. 1

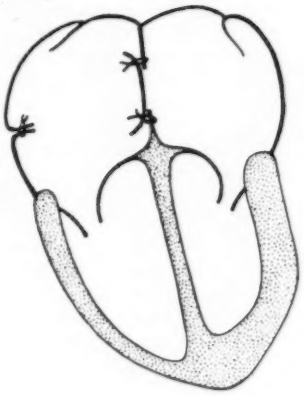


FIG. 2

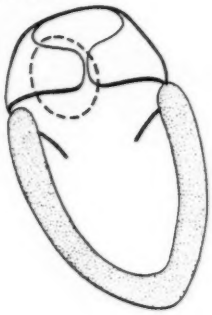


FIG. 3

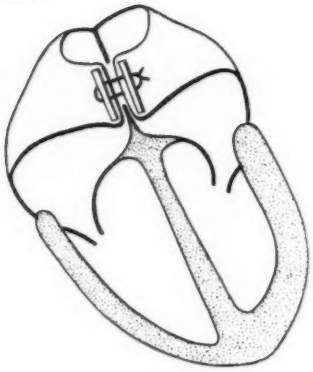


FIG. 4

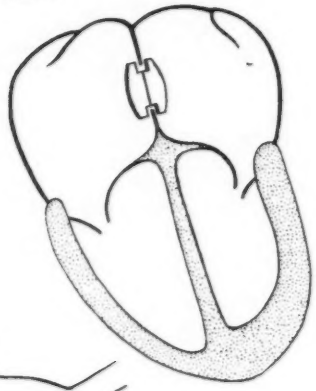


FIG. 5

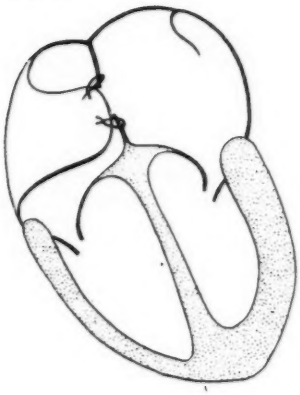


FIG. 6

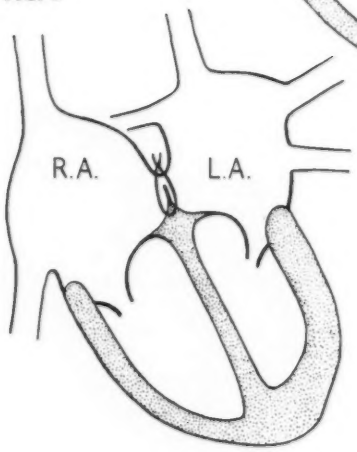


FIG. 7

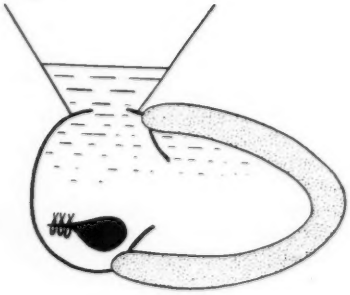


Fig. 1. Cohn's technique of closing atrial defects with a patch of atrial wall

Fig. 2. Murray's method of approximating the anterior and posterior atrial walls across the defect

Fig. 3. Swan's method of closure by invagination of both atrial appendages

Fig. 4. Plastic buttons used to occlude septal defect (Hufnagel)

Fig. 5. Bailey's method of atrio-septo-pexy

Fig. 6. Björk and Crafoord's modification of Søndergaard's technique. Groove has been dissected between atria

Fig. 7. Gross's method of closure at the bottom of a well of blood

Further features which favour a diagnosis of ostium primum are a tendency towards left ventricular hypertrophy in the electrocardiogram and the ease with which the cardiac catheter is likely to enter the left ventricle.

Persistence of the foramen ovale is a normal finding in many autopsies; it does not impose an additional burden on the heart. Its only possible disadvantage would be in allowing the passage of a paradoxical embolus.

2. Principles of Treatment

In the treatment of defects of the atrial septum, the method of choice is by suture of the defect under direct vision. An accurate anatomical diagnosis is then possible, and associated defects of the pulmonary veins and mitral valve can be identified and corrected. Much experimental work has been concerned with attempts to close large atrial septal defects with sheets of plastic or living tissue, but these are unlikely to be necessary since the edges of the defect in the large lax redundant atrium can easily be brought together without tension in the dry heart. The provision of a dry heart for direct suture poses a number of problems, so that closed and open methods of treating the condition have developed concurrently.

3. Methods of Closure

a. Closed Techniques

The pioneer work of Cohn (1947) and Murray (1948) forms the basis for the many subsequent modifications of their techniques. Cohn's method was to suture an area of atrial wall down to the defect, and this area of wall was subsequently cut free, leaving a patch of atrium over the defect (fig. 1). Murray attempted to close the defect by passing a mattress suture anteroposteriorly through the region of the septum, and this was then tied to close or reduce the size of the defect (fig. 2). This latter method depends on the blind placing of sutures in relation to predetermined landmarks on the surface of the heart. Cohn's technique was modified by Swan, Moresh, Johnson & Warner (1950), who invaginated both atrial appendages over buttons to bring them into apposition over the defect (fig. 3), and a development of this button technique was the method of Hufnagel & Gillespie (1951), in which two plastic buttons were closed over the defect and placed in position with an ingenious applicator (fig. 4). None of these methods makes provision for the establishment of an exact anatomical diagnosis, nor do they enable one to treat associated anomalies.

Bailey, Bolton, Jamison & Neptune (1953) have developed a method which is both practical and embodies most of the surgical requirements of a satisfactory closure short of direct suture. They have called the method " atrio-septo-pexy " (fig. 5). In their method, a finger is first inserted through the right atrial appendage to confirm the presence, position and size of the defect. In addition, anomalous veins can be felt and a stenosed mitral valve can be split. With the left index finger then inserted as a guide, mattress sutures are placed through the atrial wall and septal margin in such a manner as to sew a patch of atrial wall down over the defect as in the Cohn procedure. The atrial wall is left in a concave shape, without freeing the patch. By modifications of the technique, Bailey and his co-workers are also able to transpose anomalous veins into the left atrium (Neptune, Bailey & Goldberg, 1953).

Søndergaard (1952, personal communication to Björk & Crafoord, 1953) used a development of Murray's suture technique, and this has been further modified by Björk & Crafoord (1953). The latter technique involves the dissection of the tissue plane between the right and left atria and then the accurate placing of a constricting suture to bring the dissected groove down on to the interventricular septum. Again the needle tip is guided by a finger passed through the right atrial appendage. The advantage claimed for this method is that it is said to be possible to use it to close the difficult ostium primum type of defect in relation to the atrioventricular valves (fig. 6).

b. Open Methods

Gross, Watkins, Pomerans & Goldsmith (1953) have described a method of closure which involves a new concept in the surgery of the heart. Although an open method, it is still a blind procedure involving the suture of a rubber well to the wall of the atrium. An incision is then made in the atrial wall and blood rises up to a level corresponding with the pressure head of blood in that chamber. The defect is then sutured by blind palpation under the surface of the blood (fig. 7). Considerable technical skill is required but the principle of direct suture of the defect is sound.

The mechanical heart-lung in its many forms and modifications has been used extensively in experimental animals (Miller, Gibbon, Greco, Smith, Cohn & Albritten, 1953), and in a few human cases (Dennis, Spreng, Nelson, Karlson, Nelson, Thomas, Elder & Varco, 1951; Helmsworth, Clark, Kaplan & Sherman, 1953) for the closure of atrial septal defects under direct vision in a dry field. Apart from the many technical difficulties inherent in the use of this apparatus, these authors found that the coronary venous return constituted a considerable source of blood loss and hampered surgery. These machines may find their place in combination with hypothermia when the diminished circulatory requirements may allow simplification of the apparatus and add to its safety.

Hypothermia was first used to provide a dry right heart by Bigelow, Callahan & Hopps (1950), and Lewis & Taufic (1953) reported the first successful closure of an atrial septal defect in a human case. This has been followed by the report of a number of successful cases by Swan *et al.* (1953). The advantage of the method, whether induced by surface or blood-stream procedures, lies in its simplicity and the absence of complicated apparatus during the cardiac surgery. In addition, the hypothermic heart appears to tolerate manipulation of the atrial walls and septum. The disadvantages lie in the increased tendency of the ventricle to fibrillate and in the relatively short period of intracardiac surgery permitted (about 10 min.) at temperatures of about 25°–28° C. This time is adequate for straightforward atrial defects, and it offers the most acceptable method of closure at the present time.

Cross-circulation techniques (Warden *et al.* 1954) are not justified for the closure of atrial septal defects of simple type when present hypothermic techniques can be used with safety. Defects of the ostium primum type may need the extra time provided by cross-circulation.

4. Defects of the Ventricular Septum

These were formerly thought to be very common, at a time when all cases with a harsh systolic thrill and murmur to the left of the sternum were conveniently classified as maladie de Roger. With more critical assessment and modern methods of diagnosis, the incidence of pure ventricular septal defect falls to the neighbourhood of 8% of all cases of congenital heart disease (Wood *et al.* 1954). When this defect is considered as part of the complex in other conditions such as

the tetralogy of Fallot and Eisenmenger's disease, the total incidence is nearer 25%.

Attempts to deal with the defect surgically have not been as numerous as in atrial defects. There are a number of reasons for this, not the least of which has been the good prognosis that the diagnosis was thought to carry. Other factors complicating attempts at surgical correction are the difficulty of access through the ventricular muscle and the proximity of the valve rings and conducting bundle.

Attempts have been made experimentally to thread a pericardial bundle through the defect, but it is clear that an adequate closure can be obtained only with an unhurried repair of the defect under direct vision.

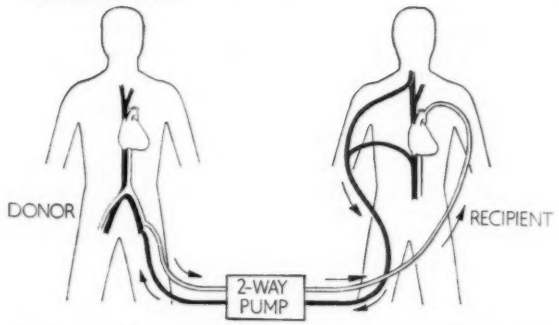
The first attempts were with the aid of the mechanical heart-lung, and Gibbon, Miller, Dobell, Engell & Voigt (1954) have described their most recent experiences with this method in dogs. Satisfactory closures have been effected by direct suture, but the clinical application of the technique, as with atrial defects, must await the development of acceptable apparatus.

Hypothermia offers a possible alternative, and Bailey, Cookson, Downing & Neptune (1954) have reported the successful closure of a ventricular defect in a young girl of 15 years. The chief disadvantage is the limited time available for what may be a technically difficult repair. Open ventricular cardiotomies are a practical possibility (Brock & Ross, 1955), and three open infundibular resections have been successfully carried out in the Thoracic Unit at Guy's Hospital. In two cases of Fallot's tetralogy with infundibular stenosis, the ventricular defect was closed in addition. In one of these, complete heart block occurred suddenly while a septal stitch was being placed; in the other a satisfactory closure was obtained, but death occurred 18 hours later from cerebral oedema consequent on too long a period of cerebral anoxia.

Increased time for intracardiac operation may be achieved by cooling to lower levels, and there has been promising experimental work in this direction. An added advantage of cooling to 10°–15° C. is the production of asystole which will facilitate the intracardiac manoeuvres.

Cross-circulation offers a prolonged period of intracardiac surgery with a non-irritable heart, and the method seems particularly adaptable to the treatment of ventricular septal defects. Lillehei has already reported a number of successful clinical cases, but possible danger to the donor must remain the chief disadvantage of the method. However, he has had no death in a donor in his first 36 ventricular cardiotomies by this method. The technique involves a principle based on azygos flow studies (Andreasen & Watson, 1952; Cohen & Lillehei, 1954), which demonstrated that dog hearts could beat satisfactorily on a greatly reduced blood flow represented by the azygos venous return only. Lillehei demonstrated that hearts can tolerate interruption of the circulation and the trauma of a cardiotomy while the coronary circulation and arterial tree is perfused with five times the azygos flow. The cross-circulation is performed by connecting the donor and recipient arterial and venous systems together by means of plastic tubing passed through a two-way pump which ensures equal flows in both directions (fig. 8). Ventricular cardiotomies have been performed on dogs at Guy's Hospital with this technique: 15 min. of exclusion of the heart has been easily obtainable in all cases, and no donor dog has died as a result of the experiment.

FIG. 8. DIAGRAM TO SHOW PRINCIPLE OF A CONTROLLED CROSS-CIRCULATION. PUMP MAINTAINS EQUAL FLOW OF BLOOD IN BOTH DIRECTIONS



5. Aorto-Pulmonary Septal Defects

This defect arises as a result of failure of complete separation of the primitive truncus into aorta and pulmonary artery. The resulting communication lies just above the valve rings and constitutes a deficiency in the walls of the vessels rather than a fistulous channel as in persistent ductus arteriosus.

Closure of the defect in this situation by ligature or suture is an extremely hazardous undertaking. Interruption of the venous inflow to the heart immediately converts the tense main arteries into lax tubes and enables clamps to be placed in position with safety for subsequent division and suture. Hypothermia meets these requirements by allowing a short period of inflow occlusion, and a defect of this nature has been closed in this manner in the Thoracic Unit at Guy's Hospital.

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SURGICAL TREATMENT IN COARCTATION

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1 Pathology
2 Clinical picture
3 Diagnosis and pre-operative investigation
4 Indications for surgical treatment
5 Operative treatment
6 Results of the operation
References

It is customary to subdivide coarctation into two types, namely, the adult and infantile varieties.

1. Pathology

In the adult form of coarctation, there is an extreme but localized narrowing which is almost invariably at the junction of the aortic arch and the descending aorta; very rarely the stricture occurs lower down the descending aorta, in which case it is most likely to be found just above the diaphragm. In the infantile type, the narrowing often extends over a much longer segment of the aortic arch, and the arterial duct, which remains widely patent, opens into the aorta below the stricture, and carries blood from the pulmonary artery into the descending aorta. The infantile type is commonly accompanied by other congenital cardiac abnormalities and usually results in death soon after birth. Although the two types differ considerably from one another it is unfortunate that they have been titled "adult" and "infantile", for it is certain that the adult form is present at birth, as it may give rise to heart failure during infancy. It is also a mistake to emphasize the extent of the narrowing as an important distinguishing feature, for the adult form is not always confined to a short segment.

Up to the present time, surgery has been almost confined to the treatment of the adult form; further discussion will therefore be limited to this type. As the aortic stricture is generally severe, the trunk and lower limbs receive their blood supply almost entirely through collateral vessels which connect branches of the subclavian arteries to the intercostal, lumbar and inferior epigastric arteries; these latter vessels carry the blood into the aorta or its branches distal to the coarctation; they are much enlarged, tortuous and often thin-walled; they may become frankly aneurysmal, especially the intercostal arteries entering the aorta just below the stenosis. In order to maintain a sufficient circulation through these collateral vessels, the blood pressure in the arteries arising proximal to the coarctation is raised, sometimes to a very marked degree, and there is consequently hypertrophy of the left ventricle. The ascending aorta is commonly enlarged, and there is frequently some dilatation of the descending aorta immediately below the stenosis; on rare occasions a saccular aneurysm is formed from the aorta, usually distal to the stricture.

Other congenital abnormalities of the cardiovascular system may complicate the adult type of coarctation. The aortic valve, which is said to be bicuspid in 25% of the cases, may show some stenosis or incompetence; subaortic stenosis is also not uncommon. The duct remains open in about 10%; it usually opens into the aorta distal to the stenosis, but the blood flows through it from the aorta into the pulmonary artery, that is, in the opposite direction to that which occurs in the infantile type: on occasion, the duct communicates with the aorta proximal to the stenosis, in which case there is gross distension of the pulmonary artery and its branches. Hypoplasia of the aortic wall has also been described. Endocardial fibro-elastosis is sometimes responsible for the early death of infants with the adult type of coarctation. Berry aneurysms of the cerebral arteries are reported to be present in 10% of the patients and, in view of the hypertension, are particularly liable to rupture. Bacterial endarteritis with vegetations at or immediately distal to the stenosis has been responsible for about one-third of the deaths resulting from coarctation.

2. Clinical Picture

Patients with coarctation of the aorta are usually well developed and may be of more than average physique. The condition is more common in males. It is most frequently recognized before the appearance of symptoms, following the chance discovery of either brachial hypertension or an unexplained systolic murmur.

Less commonly the diagnosis is not made until the patient goes to his doctor complaining of symptoms directly due to the effects of the coarctation. For example, the patient may suffer from headache or vertigo as a result of the cephalic hypertension, or abnormal fatigue and dyspnoea after exertion owing to cardiac strain. Occasionally the chief complaint is cold feet, weakness of the legs or even pain in the calves on exercise, due to the deficient circulation in the lower limbs. Exceptionally the coarctation is not detected until some complication such as heart failure, bacterial endarteritis or intracranial haemorrhage has occurred. Although children with coarctation do not often suffer any symptoms, infants with the adult type of coarctation sometimes develop heart failure, presumably because the collateral circulation is not well developed.

3. Diagnosis and Pre-Operative Investigation

Coarctation of the aorta is one of the few congenital cardiovascular abnormalities in which a diagnosis can nearly always be made by simple physical examination. A raised brachial blood pressure associated with absent or grossly diminished and delayed pulsation in the lower limb arteries is almost pathognomonic of the condition, provided that there has been no acute episode suggesting a dissecting aneurysm or a saddle embolus at the aortic bifurcation. The elevation of the brachial pressure may be quite mild in childhood, but, later in life, the systolic level is rarely below 170 mm. Hg and may be much higher. On the other hand, I have seen an infant with a systolic pressure of 230 mm. Hg which fell subsequently, presumably due to improvement in the collateral circulation.

In the normal person the blood pressure in the lower limbs is 10–20 mm. Hg higher than that in the arms: in patients with coarctation the pressure in the legs is invariably lower than that in the arms, in fact it is frequently unrecordable with the standard sphygmomanometer, because of the very small pulse pressure. This does not necessarily indicate an exceedingly low mean pressure, for direct measurement from a needle inserted into the femoral artery may show a mean pressure which approximates that of a normal person. In addition, patients with coarctation frequently show an extreme elevation of blood pressure in the arms after exercise.

Coarctation of the aorta often leads to exaggerated pulsation in the neck, and there may be a heaving impulse at the apex, due to left ventricular hypertrophy. A murmur, which is maximal in the interscapular region and loudest in systole, helps to confirm the diagnosis, but the absence of any murmur does not exclude coarctation. A continuous murmur over the base of the heart in the presence of other signs of coarctation suggests that there is patency of the arterial duct as well as coarctation. Likewise, a loud systolic bruit at the aortic base which is conducted into the arteries of the neck is evidence that the coarctation is complicated by aortic or subaortic stenosis: an aortic diastolic murmur indicates that valvular insufficiency is an additional complication.

When coarctation is suspected, the axillae, scapular muscles and abdominal wall should be examined carefully for enlarged tortuous arteries which carry the collateral circulation, but these collateral vessels often do not develop sufficiently to become visible or palpable until after puberty.

No further investigation is usually necessary to establish the diagnosis, but a chest radiograph may provide confirmatory evidence. The aortic knuckle is less prominent than normal or even absent, but there is often a bulge of the mediastinal shadow at a slightly higher level, due to dilatation of the left subclavian artery at its origin from the aorta. Also, a dilated ascending aorta is sometimes visible to the right of the sternum and, exceptionally, a post-stenotic dilated aorta may be seen descending behind the heart shadow. The cardiac silhouette may be enlarged because of left ventricular hypertrophy, particularly in adult patients; the degree of enlargement reflects to some extent the amount of cardiac strain, although Lewis (1933) states that, once compensation has been achieved, progressive enlargement does not occur. Notching of the inferior borders of some of the ribs is usually present after puberty; this is due to pressure erosion from dilated tortuous intercostal arteries: the absence of notching, especially in children, does not deny the diagnosis. Notching confined to the right side indicates that the aortic narrowing is proximal to the origin of the left subclavian artery or that the origin of the left subclavian artery is involved in the stenosis (Plate VI, fig. B, facing p. 193). Gross plethora of the lung fields probably signifies the presence of a patent duct opening into the aorta above the constriction.

Fluoroscopy during the ingestion of barium emulsion often shows two impressions on the left side of the oesophagus instead of the single one caused by the normal aortic arch.

The electrocardiogram may be normal but usually shows left ventricular hypertrophy.

Angiography is not required for diagnosis but it may demonstrate the exact site and length of the stenosis and thus indicate whether the lesion is anatomically favourable for surgical treatment. In my opinion angiography is not an essential pre-operative investigation, provided that a suitable homologous aortic graft is available in the theatre at the time of operating. If the coarctation is not shown clearly by intravenous angiography, some clinicians recommend retrograde aortography, but this is seldom justified unless the case presents some very unusual features.

4. Indications for Surgical Treatment

The prognosis of patients with coarctation has been clarified by the report of Reifenstein, Levine & Gross (1947). They found that, in 104 cases noted to have coarctation at autopsy, 26% lived a normal life-span and died of some other cause, and 74% died as a result of the coarctation (22% from bacterial endarteritis, 23% from aortic rupture and 29% as a result of the hypertensive state). The average age of those dying from the coarctation was 30, which is an indication of the serious nature of the abnormality. Against this, we must balance the risks of surgical treatment and the chance of a successful result from operation. Clagett & Jampolis (1951) report 70 cases with a mortality of 7.1%. Gross (1953) reports 270 patients with 15 deaths in the first 100, but he has had only two deaths in the last 100 cases. The operative mortality is thus considerable, even in the most expert hands. The chance of relieving the hypertensive state by operation is very good; Gross obtained complete or satisfactory relief in 98%.

It is probably right to recommend operation in all patients under the age of 40 with evidence of a really tight stricture, provided that there is no evidence of myocardial failure. Over this age, surgical treatment becomes increasingly hazardous because of degenerative changes in the walls of the aorta and of its branches in the region of the stenosis, and therefore operation should not be recommended unless there are strong indications for surgical relief. The ideal age for operation may well be about 12 to 14 years, for there is then little degenerative change in the vessel walls and it is possible to provide a lumen of such size that permanent relief from hypertension is likely, even if the lumen does not increase in size with age. Some surgeons prefer to operate on younger patients, as operative difficulties increase progressively with age, but it is questionable whether this policy is justifiable as it is not yet certain that the lumen will increase with age.

It is true that heart failure may occur in infancy and that this has precipitated operation by some surgeons, but Gross (1953) states that most of these infants recover from their failure with medical measures and subsequently develop an adequate collateral circulation.

5. Operative Treatment

There is no doubt that the ideal treatment of coarctation is resection of the entire stricture followed by end-to-end anastomosis of the divided ends. If the stenosis extends over a long segment of the aorta or if there is aneurysmal dilatation or other disease of the aorta below the stricture, excision of the affected segment may leave too long a gap to permit approximation of the aortic ends, even after full mobilization: it is then necessary to restore continuity by the insertion of a suitable homologous graft as recommended by Gross (1951) and Brock (1953). Gross (1953) reports that he has used grafts in 37 cases, with excellent or satisfactory results in 33 of the 34 survivors. He states that grafts have been necessary rarely in children but frequently in adults (23% of those operated on between 21 and 30 years). Prior to the use of

grafts, a gap which could not be bridged by primary anastomosis was often spanned by using the left subclavian artery (Blalock, 1947). After the proximal end of the aorta had been closed, the subclavian artery was divided between a proximal non-crushing clamp and a distal ligature; the artery was then turned down and an anastomosis made between it and the distal end of the aorta. Obviously this operation almost completely abolishes the collateral circulation through the branches of the left subclavian artery, and, although the subclavian artery may be considerably dilated, the brachiocephalic hypertension is often meagrely relieved by this operation which has therefore been abandoned by most surgeons.

On some occasions the aortic narrowing tapers on one or both sides of the maximal stricture, and the surgeon may be tempted to divide the aorta close to the maximal stenosis and thus avoid the use of a graft which would be necessary if the entire length of the narrowing were excised. In my opinion, a graft should be used if it is not otherwise possible to provide a lumen having a diameter of at least 70% of the optimum, for a lumen smaller than this is liable to lead to incomplete relief of the hypertension.

The operation is made far easier and safer if the blood pressure is artificially reduced by means of the drug Arfonad given intravenously by the drip method: this drug is very rapidly excreted, and so the anaesthetist can control the blood pressure at will by altering the rate of the drip. Artificial hypotension has an additional advantage in that it reduces the amount of blood loss during operation. On the other hand, severe haemorrhage may occur at some stage of the operation, so that blood should always be transfused during the procedure, and it is desirable to have about six pints[1] ready cross-matched in the theatre.

The coarctation is approached through the bed of the fifth rib which is removed from the tip of the transverse process round to the costal cartilage. In isolating the coarctation and adjacent segments of the aorta, it is advisable to start by freeing the left subclavian artery and the aorta proximal to the stricture as these rarely give rise to branches, unless the stenosis is unusually low; in this case the first intercostal vessels may arise from the aorta above the stricture. All surgeons take great care to avoid damage to the thin-walled dilated intercostal branches, but there is often also a large bronchial vessel arising from the anterior surface, which must be isolated and divided between ligatures. It is rarely necessary to divide more than two intercostal branches on each side; sometimes it is possible to place the distal aortic clamp satisfactorily without dividing any of the intercostal arteries, for these vessels run upwards and therefore do not hinder proximal mobilization of the distal aorta. When the isolation is completed, the aorta above and below the constriction is occluded with either a Crafoord or a Potts coarctation clamp: the latter is preferable if the coarctation occurs close to the origin of the left subclavian artery, as it occludes a narrower segment of the aorta.

The two ends of the aorta are approximated with a continuous everting mattress suture of 000 silk, which is interrupted at two or three equidistant points. A mattress suture is less likely to cut out than an ordinary continuous stitch, particularly when the anastomosis is made under tension or where there is hypoplasia of the aortic wall. It is customary to place the clamps horizontally and to insert the suture loosely in the posterior wall from inside the aortic lumen, subsequently drawing it tight so as to approximate the edges. If, instead, the clamps are placed vertically—that is, with the handles directed towards the ceiling—the whole circumference of the aorta may be sutured from outside the lumen so that the silk can be drawn taut seriatim as the suturing proceeds: this considerably simplifies the construction of the anastomosis. When the anastomosis has been completed the clamps are released, gently but with no particular effort to graduate the flow through the anastomosis, for the heart does not seem to be adversely affected by this sudden decrease in the peripheral resistance.

Post-operative care

No special care is required after operation. Post-operative haemorrhage is fortunately rare but the patient should be closely observed for signs of its occurrence. Anticoagulants are absolutely contra-indicated.

6. Results of the Operation

A successful anastomosis gives rise to the immediate appearance of pulsation in the arteries of the feet, which is synchronous with that in the radial arteries. The brachial blood pressure falls at once, although it may take two weeks or more before it reaches normal levels. Concurrently there is a marked rise in the pressure in the legs. Exceptionally the brachiocephalic hypertension is not completely relieved—probably owing to an inadequate lumen at the site of the anastomosis; but this cannot always be the explanation, for there are some cases in which the systolic pressure in the legs is appreciably higher than that in the arms, and yet the latter remains above normal.

The mortality of the operation has already been discussed. The proportion of cases in which surgical exploration reveals an abnormality that cannot be relieved by operation is exceedingly small, although, prior to the use of grafts in cases not amenable to primary anastomosis, the "inoperability" rate was significant (Gross, 1953; Clagett & Jampolis, 1951).

Aneurysm formation at the site of anastomosis has been described but is fortunately rare.

[1] 1 pint = 0.568 l.—Ed.

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SURGICAL TREATMENT OF PERSISTENT DUCTUS ARTERIOSUS

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1 Pathology
2 Clinical picture
3 Diagnosis and pre-operative investigation
4 Indications for surgical closure of a patent duct
5 Surgical treatment
6 Results of the operation
References

Surgical treatment of persistent ductus arteriosus must be based on a sound knowledge of the pathology and natural history of the persistently patent duct.

1. Pathology

This paper is primarily concerned with cases in which patency of the duct is the sole anomaly. The duct which remains open to compensate for some other cardiovascular lesion, such as pulmonary atresia or transposition of the main vessels, will not be discussed here. However, reference will be made to the duct which continues patent in the presence of some other cardiovascular abnormality for which it provides no compensation, for example, septal defects.

A patent duct causes part of the left ventricular output to pass needlessly into the pulmonary artery, from which it passes to the pulmonary veins and back to the left ventricle. The volume of this needless flow depends on the size of the duct and the resistance in the pulmonary circulation. It may be as much as three-quarters of the total output of the left ventricle. The left ventricle hypertrophies in proportion to the size of the flow through the duct. The pressure in the pulmonary artery may be raised because of the communication with the systemic circulation, and consequently the right ventricle also hypertrophies. The increased flow through the pulmonary circuit causes dilatation of the pulmonary artery and, frequently, of its branches also. Exceptionally one or more branches of the pulmonary artery may be truly aneurysmal.

It is important to emphasize that the flow through a duct does not depend simply on the size of the duct, for the flow through a large duct may be quite small when the pulmonary pressure is elevated by an increase in the pulmonary resistance. Indeed, on rare occasions, the pulmonary pressure exceeds that in the systemic circulation so that the flow through the duct is reversed.

Reports on the incidence of bacterial endarteritis in cases of ductal patency are conflicting (Boldero & Bedford, 1924; Shapiro & Keys, 1943), but it is probable that infection sooner or later affects between 10% and 30% of the patients. When it does occur, the vegetations appear primarily in the pulmonary end of the duct or in the adjacent part of the pulmonary artery.

2. Clinical Picture

The duct persists more frequently in females than in males, in the proportion of 3:1. It is often stated that the patients are under-sized and puny owing to a poor systemic circulation, but this is not my experience in the case of adults, although children may be slow in developing.

In childhood there are usually no symptoms, and the diagnosis follows the chance discovery of a cardiac murmur. On the other hand, if the duct is large in calibre, even young children may have pulmonary congestion causing breathlessness on exertion, or a chronic "wet" cough which is often labelled bronchitis. More often breathlessness is not noticed before adolescence and exceptionally it may be absent throughout a normal life-span. With increase of the pulmonary congestion, the patient may become orthopnoeic, and swelling of the legs and abdomen may follow later as a result of right heart failure. In the average case, cyanosis is not present unless extreme exertion leads to such elevation of the pulmonary pressure that the direction of flow in the duct is reversed. Very rarely the patient is constantly, or readily becomes, cyanosed because of a reversed flow caused by a great increase in the pulmonary resistance.

When the duct becomes infected, the patient suffers a febrile illness and sooner or later develops pleuritic pain of sudden onset due to pulmonary infarction. Symptoms of systemic embolism do not arise until a late stage of the infection.

3. Diagnosis and Pre-Operative Investigation

The finding of a continuous machinery murmur which is maximal in the second left intercostal space in a patient of normal colour almost certainly indicates patency of the duct. Corroborative evidence may be provided by the discovery of a Corrigan pulse, depression of the diastolic pressure, and signs of ventricular enlargement. A chest radiograph almost invariably shows abnormal prominence of the pulmonary artery below the aortic knuckle. Unless the duct is small in calibre, the film is likely to show some increase in the transverse diameter of the heart and enlargement of the branches of the pulmonary artery, which on fluoroscopy may show exaggerated pulsation, the so-called hilar dance. The electrocardiogram is frequently normal but it may show left ventricular hypertrophy; if there is predominant right ventricular hypertrophy, it is unlikely that an uncomplicated patent duct is the correct diagnosis.

No further investigation is required in the straightforward case, but the diagnosis may be difficult if the typical murmur is not present. For example, the diastolic fraction may be absent in very young children, but this is of little importance to the surgeon as operation is rarely contemplated at such an early age. Exceptionally the murmur is confined to systole in older patients or there may be no murmur at all, particularly if the duct is very large. Under these circumstances, if there is evidence of a left-to-right shunt, cardiac catheterization should be undertaken, as this will almost certainly show the site of the shunt, and it is sometimes possible to establish

the diagnosis with certainty by passing the tip of the catheter through the duct into the aorta. Intravenous angiocardiography is infrequently of diagnostic value. It is true that it is occasionally possible to appreciate re-opacification of the pulmonary artery when the radio-opaque material reaches the aorta, but it is unusual to be sure of this. On the other hand, there is invariably a spindle-shaped dilatation of the aorta in the region of the attachment of a patent duct (Steinberg, Grishman & Sussman, 1943), and, if this can be demonstrated by angiocardiography, the diagnosis is nearly certain. A patent duct can be demonstrated by retrograde aortography, but it is rarely justifiable to resort to this investigation.

With proper use of these ancillary methods of study, the only condition which is likely to be confused with patency of the duct is a defect in the aorto-pulmonary septum. Even this condition, which is rare, can usually be distinguished by the very loud and superficial nature of the murmur which is maximal at a lower level than that due to a duct.

4. Indications for Surgical Closure of a Patent Duct

The signs of patency of the arterial duct have been known to disappear spontaneously (Gilchrist, 1945; Benn, 1947), but this has been noted so rarely that it need not be entertained too seriously when considering surgical treatment. The mortality of operative closure of the uninfected patent duct is very small (two in my series of 93 cases), and the incidence of re-canalization is also small (one in my series). Therefore operation should be recommended in all patients with symptoms or in whom the leak is sufficiently large to produce pulmonary plethora or cardiac enlargement. Some clinicians do not advise operation for symptomless patients in whom there is evidence that the leak is small; but even these patients often suffer some anxiety from the knowledge that the lesion exists, and difficulties arise if the patient applies for life-insurance or for those forms of employment that require a medical examination before acceptance. Further, infection is just as likely to affect the small duct as the large one. Since the introduction of chemotherapy, the mortality of infective endocarditis has been reduced to 20%, and infection of the patent duct may carry an even lower mortality than infection of other lesions, for systemic embolism and valve damage occur only at a late stage of the infection. Even so, if we allow that 15% of patients with patent duct sooner or later become infected and that 15% of those infected die, there is still a mortality of 2.25% of all cases of patent duct from this cause.

It is therefore reasonable to recommend operative closure in all patients in whom the diagnosis is made, with the possible exception of (i) symptomless patients over the age of 40, with ducts insufficiently large to produce cardiac enlargement or pulmonary plethora, and (ii) patients with marked pulmonary hypertension.

The ideal age for operation is about six or seven years, although operation should be undertaken earlier if the child is adversely affected by the presence of the duct. Contrary to some reports, operation in adults has not been found by me to be more difficult or to carry a higher mortality, unless the duct has been large and caused marked secondary changes. Nevertheless, it is preferable to operate before there are even mild secondary effects.

Patients with marked pulmonary hypertension merit special consideration. If the pressure in the lesser circulation is so high that the flow in the duct is reversed, it is usually considered unwise to operate as these patients are often in irreversible heart failure. Patients with pulmonary hypertension due to a large flow through a wide duct clearly need operation, for closure of the duct will be followed by a fall in the pulmonary pressure. But when cardiac catheterization has confirmed a clinical impression that the flow through the duct is small and yet the pulmonary pressure is high, i.e. the pulmonary resistance is increased, operation is said to be dangerous and also of little value, as the pulmonary hypertension is stated to be irreversible; but Mathey, Nouaille, Vernant, Oustrières & Bouchard (1954) have shown that this is not always so. In fact, it is probably worth operating as long as there is any flow from the aorta into the pulmonary artery.

When the duct has become infected, operation should not be performed until the infection has been eradicated by appropriate chemotherapy, for immediate closure of the duct cures only about 60% of the patients (Tubbs, 1944; Touroff, 1943). As bacterial endarteritis causes a debilitating illness, it is wise to wait at least three months after the cure of the infection before operating.

A congenital defect of the aortic valve is not a contra-indication to closing a patent duct, but it is uncertain whether operation appreciably diminishes the left-to-right shunt in cases with a defect in the septum between the ventricles, for the flow through the defect may be increased by closing the duct.

In this discussion it is assumed that the surgeon has special experience in this form of surgery, for operation on the symptomless child carries an immense responsibility.

5. Surgical Treatment

During operation, hypotension induced by means of the continuous administration of Arfonad is invaluable when the duct is large as there is then much less likelihood of damaging the wall of the duct when it is occluded with clamps; but hypotension is not required unless the duct is large.

Exposure is obtained through the fourth rib bed, by means of a posterolateral incision. The rib is not removed or divided, but the periosteum is stripped from the inferior border and internal surface.

There are two different ways of exposing the duct: in one, the mediastinal pleura is divided between the vagus and phrenic nerves, and the vagus nerve with its recurrent branch is subsequently retracted backwards; in the other, the aorta and duct are exposed by incising the pleura behind the vagus which is then retracted forwards. The latter method may be quicker and easier but it has the disadvantage that the recurrent laryngeal nerve is drawn towards the pulmonary end of the duct where it is liable to be damaged if any accident occurs during the closure of this end of the duct. The former method, originally described by Gross (1939), is therefore preferred by me. The isolation of the duct should be carried out by sharp dissection with scissors, starting in the subadventitial plane over the aorta. The prolongation of the serous pericardium overlying the pulmonary end of the duct is carefully dissected from the duct and adjacent part of the pulmonary artery. If that portion of fibrous pericardium which extends upwards to the aorta on the anteromedial surface of the duct is divided with scissors, a curved clamp can be passed right round behind the duct without meeting any obstruction. When the duct is

very large or when there are reasons to suspect unusual friability or aneurysm formation in the neighbourhood of the duct, it is wise to isolate the aorta above and below the duct and to pass tape or floss silk round it before starting to dissect the duct.

The duct may be closed either by ligation or by division between clamps and by suture of the two ends. Many surgeons now divide and suture all cases, but this seems unnecessarily complicated in the straightforward case where the duct has considerable length and is not large in calibre. For such cases, permanent closure of the duct may be assured by using two floss silk ligatures, one at the aortic end which is tied first, and a second at the pulmonary end, and finally by placing a thread transfixion ligature between these ties. On the other hand, ligation should never be employed for wide ducts, for these are usually also short, and ligatures cannot be drawn tight enough to occlude the lumen without grave risk of damaging the wall. The two ends should be occluded with a Potts type of clamp, but those designed for the duct often prove to have " blades " insufficiently long for the wide duct; in this case, the Potts spoon-shaped anastomotic clamp is used for the aortic end of the duct (the concavity of the clamp being placed towards the aorta), and the angled coarctation clamp with straight " blades " is used on the pulmonary end. The duct is then divided between the clamps and the two ends are closed with two continuous sutures of 0000 silk.

6. Results of the Operation

A low pre-operative diastolic pressure is immediately corrected by occlusion of the duct. The continuous murmur also disappears at once, although a mild systolic murmur may be audible for a few months, but it is unusual to hear this after a year or two.

The branches of the pulmonary artery, if dilated before operation, diminish in calibre straight away, to a normal size in children and adolescents, but not always so in adults. There is a similar decrease in the size of the heart and of the main pulmonary artery, although adults, especially those over 30, may not show any spectacular change. It is fair to claim that patients operated on before the age of 20 are usually changed to completely normal individuals.

A recurrence of the leak between the aorta and pulmonary artery may occur even after dividing the duct but such " re-canalization " is very rare in uninfected cases (one in my series of 91 cases). An aneurysm communicating with the aorta, and sometimes with the pulmonary artery as well, may form at the site of the ligated or sutured duct, but this is singularly uncommon.

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SELECTION OF PATIENTS FOR SURGERY IN ACQUIRED HEART DISEASE

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1 Wounds of the heart
2 Massive pulmonary embolism
3 Pick's disease
4 Ischaemic heart disease
5 Mitral stenosis
6 Mitral incompetence
7 Aortic stenosis
8 Aortic incompetence
9 Tricuspid stenosis
References

1. Wounds of the Heart

Cardiac surgery may be said to have begun in 1896 when Rehn of Frankfurt successfully sutured a stab wound of the heart (Rehn, 1897). Over 200 cases of surgical repair of stab or bullet wounds of the heart were reported in the literature before 1914, with a recovery rate of about 33% (King, 1941). In the 1914–18 war the recovery rate rose to 50%, and in the Second World War the majority of cardiac wounds were successfully repaired, provided that the patient lived long enough to receive skilled surgical attention (Wood, 1949). Cardiac tamponade from haemopericardium must be relieved without delay; pericardial foreign bodies should be removed, for they often give rise to recurrent pericarditis with pain, fever and effusion; perforating stab or bullet wounds should be sutured; bullets embedded deeply in the myocardium may be left there if causing no trouble, but are probably better removed nowadays. This type of cardiac surgery was important because it proved that the heart was a robust organ, capable of withstanding major insults with remarkable fortitude. The surgeon was thus encouraged to trespass on hitherto forbidden ground.

2. Massive Pulmonary Embolism

One of the earliest planned operations on the heart or great vessels was pulmonary embolectomy (Trendelenburg, 1908a, b). The mortality rate, however, has always been very high (around 95%), and medical measures probably offer a better chance of survival; of the patients with massive pulmonary embolism who live long enough to receive skilled medical attention, about three-quarters recover. Treatment consists of the horizontal posture, avoidance of vasodilators including heat in any form, administration of oxygen, intravenous injection of Coramine (5 ml.) if the respiratory centre flags, avoidance of morphine or of any other drug that depresses the respiratory centre, injection of noradrenaline (10–30 μg./min.) by intravenous drip to maintain the blood pressure if it falls below 90 mm. Hg, intravenous injection of strophanthin (1 mg.) to support the right ventricle if it appears to be overloaded, and, above all, to prevent further thrombosis, intravenous injection of heparin (25,000 i.u.—i.e. 250 mg.), followed by prolonged anticoagulant therapy.

3. Pick's Disease

Pericardiectomy, though undertaken in isolated cases from 1910 onwards, did not become the treatment of choice for chronic constrictive pericarditis until Churchill began his series in the USA in 1929 (Churchill, 1929, 1936); the first successful reports in Great Britain appeared in the mid thirties (Roberts & Wilson, 1936; Edwards & Barlow, 1947). A heavy mortality rate, around 33% according to Holmes Sellors (1946), was at first discouraging, but with modern anaesthesia and antibiotics the present operative mortality barely exceeds 10%. Symptoms are greatly relieved and objective signs much improved in about two-thirds of cases (Evans & Jackson, 1952). Re-constriction, requiring a second operation about 10 years later, has occurred in a few instances and may be expected in about 10% of cases.

In considering whether or not to advise surgery in a particular case, and in comparing the results of medical treatment with those achieved by means of pericardiectomy, three cardinal points should be borne in mind.

i. Subjects with active subacute tuberculous pericarditis who pass into the constrictive phase in a matter of 3 to 12 months have a very poor prognosis whatever is done. In some series these patients are shown as dying while under medical treatment; in others, bolder surgery has increased the operation mortality rate. While no treatment is really satisfactory in this group, it is probably best to use medical measures, including a three-month course of streptomycin, as long as possible, in the hope that the disease will become less active, before recommending surgery. If the patient appears to be deteriorating steadily as a result of mechanical impedance to the circulation, however, pericardiectomy should be undertaken before it is too late, and must not be deferred on the grounds of tuberculous activity (Andrews, Pickering & Holmes Sellors, 1948).

ii. When patients present with oedema, ascites, a high venous pressure and great enlargement of the liver, without any evidence of activity, i.e. with the classic picture of constrictive pericarditis, surgical treatment should be recommended as soon as maximum improvement has been achieved by medical means. These patients do well surgically, and although they may be kept alive medically for many years, they are not comfortable citizens.

iii. An appreciable number of cases are symptom-free when first recognized and may remain so indefinitely. Surgical treatment of these might be claimed as giving good results, but the operation is pointless, for it does not cure the disease, and the physiology of the circulation is content without it.

The matter may be summed up by stating that pericardiectomy should be undertaken in all cases of chronic constrictive pericarditis (at least up to the age of 65) that would otherwise require medical treatment (apart from digitalis in cases with auricular fibrillation), but should be deferred as long as

possible when tuberculosis is clearly active. It may be added that one of the major difficulties is diagnostic, for nearly all the clinical, radiological, electrocardiographic and physiological signs of Pick's disease may be presented by cases of isolated myocarditis and other primary cardiopathies—including the steep *y* descent of the jugular pulse, Kussmaul's sign (elevation of the jugular venous pressure on inspiration), a quiet cardiac impulse, diastolic gallop, relatively still heart shadow fluoroscopically, rather low-voltage electrocardiogram with simple inversion of the T waves in most leads, considerable elevation of both left and right atrial pressures, dip and plateau appearance in the right ventricular pressure tracing, and a low cardiac output. The only absolutely pathognomonic sign of Pick's disease under the appropriate clinical circumstances is pericardial calcification; helpful evidence against Pick's disease is more than moderate cardiac enlargement, vigorous cardiac pulsation, and left bundle branch block. When in doubt, thoracotomy may be justified.

4. Ischaemic Heart Disease

Historically, the fourth type of cardiac operation to be undertaken was designed to relieve cardiac pain by promoting some sort of collateral circulation to the myocardium (Beck, 1935; O'Shaughnessy, 1936). Twenty years later, operations of this type have not yet graduated from the experimental class.

Bilateral stellate and upper dorsal ganglionectomy, however, still has a place in the treatment of intractable angina pectoris (White & Bland, 1948).

5. Mitral Stenosis

Although the first successful digital mitral valvotomy was performed by Souttar in 1925, the operation did not gain favour until reintroduced by Harken, Ware & Norman (1948) and by Bailey (1949) in the USA, and independently by Brock in England (Baker, Brock & Campbell, 1950). Since then thousands of cases of mitral stenosis have been successfully relieved of their stricture, and parallel medical studies have placed selection of cases on a firm scientific footing. The operative mortality is around 5%, and results are excellent or good in three-quarters of the patients operated on; of the remainder, 10% are improved and a like number unchanged or worse. The incidence of re-stenosis will not be known for some 20 years, but so far it is about 2% per annum.

In general, any patient who is suffering from the effects of mitral stenosis requires valvotomy; and any patient with mitral stenosis who is able to carry on his normal occupation without distress does not; but these two statements need tempering.

In view of the obvious tendency to post-operative re-stenosis as the years go by (curiously denied in some quarters), and because the second valvotomy is proving more difficult than the first, to say nothing of the undesirability of repeated operations from the patient's point of view, premature valvotomy must be avoided. If effort intolerance is divided into four grades of severity—slight, moderate, considerable and gross, the fourth grade being total incapacity—patients with only slight effort dyspnoea (or none at all) should be left alone, and patients with considerable or gross effort intolerance should be treated surgically, the decision in respect of cases with moderate dyspnoea alone presenting any difficulty. To clarify the situation, the four grades of effort intolerance may be defined as follows:

Grade 1. Only physical activity out of the ordinary induces symptoms: e.g., running, hurrying, walking up hills, or strenuous manual labour.

Grade 2. Ordinary physical activity causes symptoms: e.g., walking any distance at an average pace, carrying a shopping basket, or doing heavy housework.

Grade 3. Less than ordinary physical activity causes symptoms: e.g., walking slowly on the level, or doing light housework. Patients in this group are usually orthopnoeic or complain of undue fatigue and oedema.

Grade 4. Total incapacity.

Grade 2 may be divided into grades 2A and 2B according to whether effort intolerance is nearer that in grade 1 or grade 3 respectively, for there are no sharp divisions between the grades. As a rule, patients in grade 2A are deferred and patients in grade 2B are advised to have a valvotomy, especially if they are deteriorating.

The following factors also affect the decision to operate or not in a given case.

Age. Patients under 20 should be deferred as long as possible in view of their presumably increased chances of re-stenosis; at the other end of the scale, when a patient is in the late fifties, it may be wise to recommend valvotomy before he reaches the age of 60, if he is already in grade 2 and deteriorating.

Activity. Although active rheumatic carditis is obviously an adverse factor, it must not stand in the way of valvotomy if life is threatened by a mechanical stricture.

Bronchitis and emphysema. Winter bronchitis is a common complication of mitral stenosis, and from time to time cases are seen in which bronchospasm and emphysema seem to have reached a stage that is unlikely to be relieved by mitral valvotomy. In fact, however, this view is rarely justified if the stenosis is tight enough to demand relief in its own right. In doubtful cases the degree of emphysema should be estimated by lung function tests, and the degree of stenosis by cardiac catheterization. Relief of bronchospasm and winter bronchitis may be expected from technically successful valvotomy.

Embolism. Systemic embolism is the one complication of mitral stenosis which calls for valvotomy at a time when the operation would be judged premature on other grounds. The reasons for this attitude are that emboli are usually recurrent and serious, that post-operative emboli are gratifyingly rare, and that the risk of embolism during the operation is little if at all higher in these patients than in those who have never had an embolism (Wood, 1954).

Haemoptysis. Sudden, unexpected, often profuse haemorrhage, due to rupture of a pulmonary or bronchopulmonary venous radicle, is apt to occur at a relatively early stage in the life-history of mitral stenosis. It usually means that the pulmonary venous pressure is beginning to rise briskly on effort, that the delicate pulmonary and bronchopulmonary veins have not yet thickened in response to this rise of pressure, and that the pulmonary vascular resistance is low (Wood, 1954). In view of this and since it is very rarely dangerous in itself, pulmonary apoplexy should not ordinarily be regarded as an indication for valvotomy. Recurrent bleedings may be discouraged by a low-sodium diet and temporary restriction of physical activities. After a year or two they are likely to cease spontaneously.

Pulmonary oedema. When the pulmonary vascular resistance is low, and the valve orifice constricted to the critical size of around 10 mm. × 5 mm., attacks of pulmonary oedema may endanger life. With an unusually low resistance and some factor tending to increase the cardiac output, such as pregnancy, anaemia, or some febrile illness (including rheumatic fever with or without carditis), attacks may occur when the stenosis is relatively mild (orifice approximately 15 mm. × 7.5 mm.). Toxaemia of pregnancy is a particularly dangerous association. Pulmonary oedema demands urgent valvotomy, but the physiological situation should be controlled first by means of rest, posture, sedatives, and dehydration (mercurial diuretics and a rigid low-sodium diet). Pulmonary oedema is an acute medical emergency, but it is not an acute surgical emergency.

An extreme pulmonary vascular resistance. In uncomplicated cases of mitral stenosis severe enough to warrant valvotomy, the mean left atrial pressure at rest averages about 22 mm. Hg above the sternal angle, the mean pulmonary artery pressure 10–20 mm. Hg higher, the cardiac output 4–4.5 l./min., and the pulmonary vascular resistance 2–4 units (160–320 dynes sec./cm.^{5}). In about 20% of all cases of mitral valve disease the resistance is high (above 6 units), and in about 7.5% extreme (10–30 units). These are the cases that sooner or later develop a large right ventricle and congestive heart failure, and all require valvotomy whatever their grade of effort intolerance, for high resistances do not develop unless the degree of stenosis is critical (10 mm. × 5 mm.), and the protection from pulmonary venous congestion afforded by pulmonary vasoconstriction tends to prevent dyspnoea. Cases with right ventricular failure, whether improved by medical treatment or not, run a grave risk of phlebothrombosis and fatal pulmonary embolism; anticoagulants should be given at once to this group and continued until valvotomy can be undertaken. No case of this kind is too advanced for surgical treatment, although the best results cannot be expected if this is left too late. Irreversible pulmonary hypertension has not yet been encountered.

Cardiac catheterization offers direct proof of a high pulmonary vascular resistance, but indirect evidence is usually sufficient: this includes absence of paroxysmal cardiac dyspnoea and of recent pulmonary apoplexy, symptoms and signs of right ventricular failure with normal rhythm or controlled auricular fibrillation, a florid mitral facies and other evidence of an unusually low cardiac output, a strong *a* wave in the jugular pulse if there is normal rhythm, a heaving thrust over the right ventricle, damped auscultatory signs of mitral stenosis, sharp accentuation of the pulmonary element of the second sound, pulmonary incompetence, functional tricuspid incompetence, right ventricular preponderance on the electrocardiogram and conspicuous dilatation of the pulmonary artery radiologically.

Auricular fibrillation. The presence or absence of auricular fibrillation has no bearing on the question whether or not a case of mitral stenosis requires valvotomy. Auricular fibrillation is related more to the age of the patient than to any other factor. When the ventricular rate is uncontrolled it may cause intense pulmonary venous congestion (owing to impaired diastolic left ventricular filling) or congestive heart failure with oedema, and may thus give a totally false impression of the severity of the stenosis or the degree of pulmonary vasoconstriction. When the stenosis is relatively mild, total incapacity from uncontrolled auricular fibrillation may be replaced by only grade 1 effort intolerance after digitalis therapy, or after restoration of normal rhythm by means of quinidine.

Physical Signs

The only unequivocal physical signs of mitral stenosis itself are auscultatory, and consist of the presystolic and diastolic mitral murmurs, the loud first sound and the opening snap. Of these, only the length of the diastolic murmur and the closeness of the snap to aortic valve closure offer any real indication of the degree of stenosis. In relatively mild cases the murmur stops half way through diastole, and the snap is late (0.1–0.12 sec. after aortic valve closure). The chief auscultatory difficulties or misinterpretations are (i) the remarkable damping of all mitral stenotic phenomena in cases with an extreme pulmonary vascular resistance (even to total disappearance of all auscultatory signs), probably caused by posterior displacement of the small left ventricle; (ii) damping of the first heart sound and disappearance of the opening snap in calcific cases (Wynn, 1953); and (iii) the development of a loud tricuspid systolic murmur at the " mitral area " in cases of functional tricuspid incompetence secondary to gross pulmonary hypertension—a murmur which is often mistaken for that of mitral incompetence.

The Electrocardiogram

Where there is normal rhythm it can be stated confidently that no case of mitral stenosis is severe enough to warrant valvotomy unless there is a P mitrale in the electrocardiogram (excluding embolic cases). A second good rule is that right ventricular preponderance guarantees sufficiently tight stenosis to justify valvotomy, for a high pulmonary vascular resistance does not develop in mild cases. A third equally good rule is that abnormal left ventricular preponderance means dominant mitral incompetence (assuming there is no aortic valve disease or hypertension) and so contra-indicates valvotomy.

Radiological Appearances

Too great a degree of cardiac enlargement is often invoked as a reason why valvotomy should not be undertaken, yet the size of the heart means little unless the dilated chambers are identified. Thus aneurysmal dilatation of the left atrium is no contra-indication to surgery if it can be shown that mitral incompetence is not responsible; a greatly dilated pulmonary artery, right ventricle and right atrium mean an extreme pulmonary vascular resistance, and this makes valvotomy imperative, however great the enlargement; a much distended right atrium without enlargement of the pulmonary artery usually means tricuspid stenosis and this is no bar to mitral valvotomy; only when the enlargement is due mainly to dilatation of the left ventricle should it halt surgical arrangements, and then only if mitral or aortic incompetence can be held culpable.

Radiographs usually provide good evidence concerning the physiological situation in cases of mitral stenosis. Thus the degrees of pulmonary venous congestion and lymphatic engorgement are directly related to the pulmonary venous pressure and therefore to the degree of stenosis; but they are also inversely proportional to the pulmonary vascular resistance. If the pulmonary artery and right atrium are strictly normal, indicating a normal resistance and absence of tricuspid stenosis respectively, clear lungs are incompatible with serious mitral stricture.

Mitral Incompetence

The chief practical difficulty in selecting cases suitable for valvotomy consists in recognizing and vetoing all those with too great a degree of mitral incompetence. In view of their great importance in this respect, the chief signs of serious mitral incompetence are listed below:

i. a small, somewhat collapsing, peripheral pulse in the absence of aortic incompetence and hepatic failure;
ii. a raised jugular venous pressure when the pulmonary vascular resistance is not high (judged clinically), when the ventricular rate is more or less normal, and when there is no evidence of tricuspid stenosis or pericardial effusion;
iii. a hyperdynamic left ventricular type of cardiac impulse when there is no aortic incompetence;
iv. absence of a presystolic murmur when there is normal rhythm;
v. a soft, normal or slightly accentuated first heart sound when there is no mitral calcification;
vi. a loud mitral pansystolic murmur with or without thrill, which must not be confused with an aortic or tricuspid systolic murmur heard best at the mitral area;
vii. absence of an opening snap when there is no mitral calcification;
viii. a loud third heart sound;
ix. a relatively short mitral diastolic murmur;
x. an electrocardiogram showing left ventricular preponderance;
xi. radiological evidence of a dilated, hyperdynamic, rapidly filled left ventricle (typically associated with considerable enlargement of the left atrium, a relatively normal pulmonary artery, and only slight pulmonary venous congestion);
xii. in cases of gross incompetence, obvious expansile pulsation of the left atrium in the anterior view is highly characteristic;
xiii. a direct or indirect left atrial pressure pulse showing a rapid *y* descent or an R*y*:*v* ratio measuring more than 1.6 (see later).

In difficult border-line cases, heavy calcification of the mitral valve is common. When stenosis is dominant, partial valvotomy (usually of the lateral commissure) may be worth while, but an over-zealous surgeon may easily aggravate the incompetence, especially when struggling with a resistant medial commissure.

Cardiac Catheterization

By wedging a catheter in a distal branch of the pulmonary artery as described by Lagerlöf & Werkö (1949), the left atrial pressure pulse may be accurately recorded (Epps & Adler, 1953). In mitral stenosis the mean left atrial pressure is raised, averaging 22.5 mm. Hg above the sternal angle in cases severe enough to warrant valvotomy, the range being 10–35 mm. Hg at rest in this group. Since the left atrial pressure in any case of mitral stenosis varies directly with the cardiac output, the latter should be estimated simultaneously. The simple index

$$\frac{\text{cardiac output (l./min.)}}{\text{left atrial pressure (mm. Hg)}} \times 100$$

gives a good idea of the degree of stricture, figures of 10–15% being found with extreme stenosis (orifice around 5 mm. × 3 mm.), about 20% with average critical stenosis (10 mm. × 5 mm.), and around 33% when the stenosis is less than usual but still tight enough to warrant valvotomy if the symptoms demand it. In mild cases with only slight, if any, effort intolerance, the atrial left pressure is under 10 mm. Hg in relation to the sternal angle, and the mitral index 50–100%. These figures are of value only when the degree of mitral stenosis is being questioned, and become invalid when there is more than trivial mitral incompetence.

To assess the degree of obstruction to forward flow, Owen & Wood (1955) measured the speed at which the left atrial pressure fell after the mitral valve opened, for the slower the *y* descent the greater the obstruction. Since the rate of the *y* descent was also related to the height of the *v* wave, the ratio R*y*:*v* gave a more reliable idea of the degree of obstruction than the rate of *y* descent (R*y*) alone. If R*y* is expressed in millimetres per second, and *v* in millimetres above the sternal angle, R*y*:*v* should be more than 1.6 if there is no significant obstruction to forward flow. In extreme stenosis R*y*:*v* is less than unity, and with average critical stenosis (orifice 10 mm. × 5 mm.) it is usually about 1.2. The value of this ratio has proved itself over and over again in difficult cases with combined stenosis and incompetence; it also distinguishes mitral stenosis from left ventricular failure in cases with combined aortic and mitral valve disease.

The third object of cardiac catheterization is to measure the pulmonary vascular resistance:

$$R = \frac{\text{mean } PAP - \text{mean } LAP}{\text{pulmonary blood flow (l./min.)}}$$

where *R* is the resistance expressed in simple units, *PAP* is the pulmonary artery pressure in millimetres of Hg, and *LAP* is the left atrial pressure in millimetres of Hg. The normal pulmonary vascular resistance is 1–2 units.

Three grades of increased resistance are recognized: slight at 3–5 units, clinically important at 6–9 units, and extreme at 10–30 units. Cases in the extreme grade may be mistaken clinically for primary pulmonary hypertension, the signs of mitral stenosis being masked. The high left atrial pressure and the form of the left atrial pressure pulse (low R*y*:*v* ratio) proves that mitral stenosis is responsible.

To sum up, cardiac catheterization may be required to prove whether a case of mitral stenosis is severe enough to warrant valvotomy when the clinical features are indecisive, to distinguish dominant mitral stenosis from incompetence or left ventricular failure, and to demonstrate the cause of extreme pulmonary hypertension when the aetiology is clinically in doubt. There is no object in catheterizing straightforward cases of mitral stenosis in which problems of this kind do not arise.

6. Mitral Incompetence

Although many attempts have been made to repair mitral incompetence (see, for example, Logan & Turner, 1952), none has been really successful so far.

7. Aortic Stenosis

Aortic valvotomy has been carried out with increasing frequency during the past two to three years, since first

reported by Bailey, Redundo-Ramirez & Larzeleke (1952). The early high mortality rate (33–50%) was due partly to the fact that only patients in a very advanced stage of the disease were submitted for operation, severe angina and intractable heart failure being the original criteria on which cases were selected. At present the mortality rate is still high at 22% (Bailey, Bolton, Jamison & Nichols, 1954) and is probably nearer 30% in most clinics, but as a rule very advanced cases are still being selected. From the physician's point of view the difficulty is to recommend what appears to be a dangerous operation to a patient who is virtually free from symptoms, especially when he knows that aortic valvotomy cannot cure the diseased valve, and in some cases at least may be little more than a palliative procedure. Nevertheless, if aortic valvotomy is to be of any real value, a determined and bold attack must be made at a time when the patient's condition is still relatively good, and the myocardium healthy. The criteria suggested here are angina pectoris, syncope or moderate dyspnoea when such symptoms can be attributed confidently to the stenosis; an unquestionable anacrotic pulse or a pulsus bisferiens in which the tidal wave is taller than the percussion wave; sufficient delay in aortic valve closure to cause reversed splitting of the second heart sound; obvious enlargement of the left ventricle radiologically, and considerable left ventricular preponderance with T wave inversion electrocardiographically. Physiological studies should reveal a left ventricular systolic pressure at least 20 mm. Hg higher than the arterial systolic pressure, and a resting cardiac output not exceeding 5 l./min. The left ventricular pressure may be recorded by threading a fine polythene tube through a needle inserted into the left atrium through the posterior thoracic wall just to the right of the spine at the level of the eighth intercostal space (Björk, Blakemore & Malmström, 1954).

8. Aortic Incompetence

Aortic incompetence may be treated surgically by inserting a plastic valve in the descending aorta as described by Hufnagel, Harvey, Rabil & McDermott (1954). This operation is still in the experimental stage and at present can be considered only in advanced cases. There were six deaths in the series of 23 cases reported, but the 17 survivors were said to be all greatly improved.

9. Tricuspid Stenosis

About 3% of all cases of mitral stenosis also have tricuspid stenosis, and of these 5–10% are probably severe enough to warrant tricuspid valvotomy (Gibson & Wood, 1955). In addition to the inspiratory tricuspid presystolic and diastolic murmurs, which give little indication of the degree of stricture, there should be a giant *a* wave in the jugular pulse if there is normal rhythm, a high *v* wave with gentle *y* descent if there is atrial fibrillation, a quiet right ventricle, little dilatation of the pulmonary artery, considerable dilatation of the right atrium, little or no right ventricular preponderance in the electrocardiogram, and, when there is normal rhythm, tall P waves measuring 3–5 mm. in height. Fatigue, hepatic enlargement, ascites and oedema are characteristic. Cardiac catheterization should confirm the relatively slow rate of *y* descent in proportion to the height of the *v* wave, and demonstrate a diastolic pressure gradient across the tricuspid valve of at least 5 mm. Hg; the cardiac output at rest should not exceed 4 l./min.; and the arteriovenous oxygen difference should be well over 50 ml./l. More precise criteria on which to recommend tricuspid valvotomy cannot be laid down until sufficient experience accumulates.

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OPERATIVE TREATMENT FOR MITRAL STENOSIS

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1 General principles
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- *e* Selection of patients

2 Treatment
- *a* Pre-operative preparation
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3 Results

The successful treatment of mitral stenosis by surgery has led to a greatly increased interest in heart disease as a whole. More precise diagnosis and observation have become necessary adjuncts to the selection of suitable cases, and as a result every aspect of mechanical heart disorders has benefited.

1. General Principles

The problem of the straightforward case of " pure " mitral stenosis is fundamentally not difficult. If the stenosis can be safely overcome, considerable improvement can be expected, but difficulties arise when the picture is complicated by additional valve lesions or by secondary changes where mitral valvotomy may only relieve a part of the disability.

Mitral stenosis[1] is invariably the result of rheumatic infection, and the endocardial inflammation may ultimately lead to the narrowing of the mitral orifice with the restriction of the passage of blood from auricle to ventricle. Narrowing or impairment of valve function can exist without causing the symptoms of stenosis, which occur only when the narrowing has reached certain limits. With stenosis the normal efficient non-return action of the valve is disturbed, and reflux or regurgitation is potentially associated with the narrowed and rigid orifice. One of the problems of valvotomy is to relieve the stenosis without producing a dangerous degree of regurgitation.

a. Anatomy and Pathology

Surgical experience has added considerably to our knowledge of the normal and abnormal mitral valve. The valve itself consists of several components—the cusps, the chordae tendineae and papillary muscles—and though the cusps are the most important the other structures cannot be ignored. The cusps are attached circumferentially to the valve ring, with free edges which lie in a posteromedial and anterolateral plane. The total area of the cusps is greater than the cross-section of the mitral ring, so that there is a considerable degree of " slack " which allows the cusps to sail or float backwards and forwards. The aortic or anteromedial cusp is considerably larger than its posterolateral fellow and consequently has a greater range of movement. When the valve opens, the cusps sail into the ventricle to leave a wide open passage, but on closure the cusps come together and are prevented from being blown into the auricle by the attachment of the chordae tendineae at the free margins. The looseness or slack of the cusps causes the valve to close not only by linear approximation but by additional pressure such as occurs when the mouth is closed and the lips then pursed together.

The chordae are arranged in two fan-like systems, each arising from a papillary muscle and being inserted into the edges of both cusps. Sir Russell Brock has stressed the importance of the stout chordae which form the boundaries of the fan. These he refers to as the tendons of critical insertion and he regards them as marking the boundaries of a central blood channel, which is the effective passage, with the more peripheral chordae acting as supporting structures.

When rheumatic vegetations attack the mitral valve they affect not only the free edges of the cusps but also the chordae. Surgical experience does not suggest that stenosis develops as a gradual centripetal cicatrization of the valve; it is more usual for the edges of the cusps to become adherent (probably in the region of the chordae of critical insertion), and, though leaving an adequate central blood channel, the supporting areas or commissures are approximated and fused. The function of the valve as such may thus be lost early on in the history of rheumatic infection, but, as long as there is enough space for the passage of enough blood, few ill-effects will be noted. In the course of years fibrotic changes narrow the blood channel and produce the obstructive factors that are characteristic of the disease. Ulceration of the cusps or severe cicatrization of the chordae add to the distortion of simple stenosis. It is inevitable that with every non-functioning valve which is converted into a rigid ostium a certain amount of reflux from the ventricle occurs. This regurgitation is not significant in most cases, but becomes clinically obvious where ulceration has destroyed valve substance and left an opening that is strictly not a stenosis. Uneven cicatrization of the chordae may lead to pulling down one cusp and allowing a reflux jet to occur horizontally rather than vertically. At operation it is easy to prove this point by pressing on one or other cusp, particularly at the posterior end, and noting the regurgitation which disappears when pressure is released. One of the most audible reflux murmurs is heard when the posterior chordae to the posterolateral cusp are contracted and a small but violent jet of blood impinges on the lateral wall of the auricle.

The operation of valvotomy consists of an endeavour to split or cut open the commissures or lines of fusion between the cusps and thus restore some semblance of a normal valve. In the simple case this can be done and an orifice of full width re-established; moreover, if the cusps still bulge normally or have sufficient slack, an acting valve can be obtained. This, however, is rarely as satisfactory as expected, because the free edges of the cusps have become sclerosed and indurated like a bow-string and cannot float freely into the

[1] See Wood, p. 203 of this number of the Bulletin.—Ed.

ventricle. When there is considerable fibrosis and even calcification, enlargement of the stenosis along the line of the commissures gives a much wider blood channel, but the rigidity of the cusps is the limiting factor for valve function. Extreme fibrosis of the cusps and chordae reduces bulging and converts the valve into a rigid cone at the apex of which is a small fish-mouth opening which can be slightly dilated but not divided. Cross-fusion of the chordae may make a secondary stenosis below the level of the cusps and thus add to the problems of operation.

The size of the stenotic orifice in the average case is remarkably constant in the absence of obvious regurgitation. There is some variation in relation to the size of the mitral ring, but 1.0 cm. × 0.5 cm. to 1.25 cm. × 0.75 cm. would cover the majority. Though there may be clinical justification for the term " tight " mitral stenosis, there is little anatomical evidence for differentiation between this and any other form of established stenosis. There appears to be a critical point at which the primary symptoms are initiated, and even if only slight improvement is produced by operation considerable benefit may result.

b. Circulatory Mechanics

The basic feature of stenosis is inadequate flow into the left ventricle and a steady building-up of pressure in the left auricle, which is referred directly on to the pulmonary veins, thence via the capillaries on to the pulmonary arteries. Ultimately the right ventricle hypertrophies, and a progressively raised pulmonary artery pressure develops. The raised pressure in the pulmonary circulation is an essential feature of the disease and determines a great deal of the symptomatology. A capillary pressure which is higher than the cellular osmotic pressure may well lead to pulmonary oedema, and, in the case of high pulmonary artery pressure, there must be some mechanism such as a protective arteriole spasm that prevents a permanent state of pulmonary oedema. Advanced pulmonary hypertension will sooner or later reflect itself on the myocardium and contribute to congestive heart failure.

Beyond the stenosis the left ventricle, deprived of adequate blood filling, is not enlarged unless there is the factor of regurgitation to be considered. The aorta is similarly small, and the paucity of an adequate systemic flow is reflected in the patient's nutrition. The splendid physique of patients suffering from coarctation of the aorta and a resultant large aortic arch can be contrasted with that of patients suffering from mitral stenosis.

The state of the myocardium as the result of rheumatic infection has been rather overshadowed by the attention directed to the valve. In former years this was not so, and myocardial damage was used as an argument against surgery: an attitude which delayed mitral surgery by a number of years.

c. Signs and Symptoms

The dominant feature of mitral stenosis is shortness of breath; this usually comes on gradually and patients may not notice their increasing disability. They find it difficult to climb stairs or to walk up slopes and later to perform the heavier type of household duties. In some cases this gradual onset is not noticed, and patients develop sudden acute dyspnoea which may take the form of pulmonary oedema. Paroxysms of dyspnoea may be provoked by emotion or exertion and are associated with moist sounds in the chest, cough and blood-stained sputum. The most severe type of attack may occur at night and may be fatal. Associated with the shortness of breath is some degree of cyanosis, and many patients exhibit the characteristic malar flush. Most patients are thin and somewhat distressed in appearance, with cold extremities and a pinched look. Cough associated with bronchitis is common and is very marked when pulmonary oedema occurs. At night the patients find they cannot sleep flat and require an increasing number of pillows to bring them into a raised position. The pulse is small, and the patient's whole nutrition bears the stamp of a small aorta. These features may be added to by the appearance of congestive failure, when moist sounds in the chest, swelling of the ankles, enlargement of the liver and increasing cyanosis appear.

On examination of the heart there is a characteristic thrust to the left of the sternum, evidenced by the enlarged right ventricle, and on auscultation the first sound is snapping and is usually preceded by rumbling diastolic murmur. This presystolic sound leads up to the first sound with a marked intensity, and over the base of the heart a loud second sound can be heard associated with an opening snap whenever the valve is of the mobile diaphragm type. Auricular fibrillation is commonly present, and when it occurs the presystolic murmur is absent.

d. Radiological Findings

The heart is usually enlarged and of rather square shape; the aortic knuckle is small, and immediately below it is an enlarged curve of the pulmonary artery. Between this and the left border of the heart there is often a subsidiary curve which has now been demonstrated as being due to enlargement of the left auricular appendix. The lung fields are engorged, and in advanced cases show signs of haemosiderosis. In the presence of heart failure the whole outline of the heart is increased, and in the majority of cases with which surgery is concerned the cardio-thoracic ratio is between 55% and 65%.

e. Selection of Patients

The problem of selection of patients for operation is by no means easy and a variable number of factors have to be taken into consideration. In the first place, the patient should be neither too old nor too young; an arbitrary limit between the ages of 20 and 50 years is usually accepted. The most important point is to ensure that as long an interval as possible will have elapsed between the last signs of rheumatic activity and operation. Persistent tachycardia, raised sedimentation rate and fever are among the points to be watched. It is not necessary that the heart rhythm should be regular and in nearly a half of the operation cases auricular fibrillation is present, but heart size is important in that it reflects to some extent damage to the heart muscle or the presence of other valve lesions.

If aortic stenosis or regurgitation is present the left ventricle may well be enlarged, but this is not significant if the aortic damage is only slight. Mitral regurgitation constitutes a real problem; many cases of stenosis exist in association with regurgitation, and here the problem is to decide on the extent of this reflux. This can often be assessed only at operation.

Auricular thrombosis with embolism is a threat which hangs over patients with fibrillation, and the history of embolic episodes constitutes an indication for operation in the hope that the improved circulation will reduce the liability to this complication. Firm " white " organized clot within the heart is not dangerous, but granular " red " and friable clot

of recent formation can easily be broken off into the circulation.

In short, the ideal case for mitral valvotomy should be a young patient with a small heart in regular rhythm and with no other valve lesion, but this is the counsel of perfection and the choice has to be made on the individual merits of each case.

2. Treatment

a. Pre-Operative Preparation

Mitral stenosis does not often constitute a surgical emergency, and plenty of time can be given for preparation of the patient. With any signs of congestive failure active medical measures have to be taken to reduce the load on the heart. An irregular rhythm has to be brought under control, though it is rarely wise to try and restore sinus rhythm from fibrillation before operation. A period of rest in bed is advisable, but if there is any suspicion of auricular thrombosis and embolism the use of anticoagulants may be considered. Not least in the preparation is the psychological approach: many patients who are highly unsuitable are most persistent in their demands for surgery. The answer should be: " If the operation has any chance of improving your condition, it will be seriously considered ".

b. Anaesthesia

Considerable care has to be taken in the induction and maintenance of anaesthesia. With patients who are likely to develop paroxysmal dyspnoea they should be brought to the operating theatre well propped-up and the anaesthetic induction undertaken in this position. After many trials with drugs to reduce the irritability of the heart, the anaesthetic agents have been reduced to simple forms. Endotracheal gas and oxygen, with a minimal amount of basal narcotic and muscle relaxant, is adequate in most cases, and the absence of inflammatory agents allows the surgeon to make use of diathermy.

c. Incision

The chest is entered in the line of the fifth rib or fifth space and the incision can be made anterolaterally or posterolaterally. A wide exposure is wise, so as to allow the surgeon to deal with any untoward eventuality. When the chest is opened the lung is retracted backwards and the pericardium exposed. A longitudinal incision is made through the pericardium just in front of or just behind the phrenic nerve, and the edges of the pericardium retracted. This exposes the left border of the heart with the enlarged left auricle and appendix. The curved pulmonary artery is dilated and under high tension, and the pulmonary veins are grossly engorged.

The pulsation and movement of the auricle are observed to see if there is any evidence of clot, in which case the anaesthetist prepares to compress the carotid arteries in the neck during any manipulation which might dislodge an embolus. If the auricle is free of clot, a special narrow-bladed clamp is placed across the base of the appendix and beyond this an incision is made. It is usual to amputate the tip of the appendix and refer it for microscopical study.

The opening in the appendix should be made of a length that is one-and-a-half times the diameter of the surgeon's index finger. As the endocardium is exposed, columnae carneae may be found crossing the line of the incision and have to be divided. The control clamp is then momentarily released to drive out any clot that may be present in the auricle, and the index finger is inserted into the incision and down into the chamber of the auricle itself. The close fit of the appendix walls round the finger prevents loss of blood. The finger is passed downwards and forwards and sweeps over the curve of the valve surface at whose periphery a slightly raised edge of the mitral ring may be felt. The opening is recognized as a small roughened area at the centre of which is the actual orifice, but, before the actual valvotomy is attempted, the remainder of the valves should be palpated, when it will be found that in a case with a pliant diaphragm there is an appreciable bulge backwards and forwards in the anterior part of the aortic cusp. Suppression of the bulge by finger pressure abolishes the " opening snap ". While the finger lies within the chamber, note should be taken of any jet or stream which would indicate reflux or regurgitation through the valve.

The actual valvotomy consists of engaging the finger tip in the stenotic opening and forcibly pressing anterolaterally and posteromedially to rupture the commissures. The edge of the stenosis is thickened and often firm, resisting the forcible efforts of the finger to tear it; in such a case the use of a knife introduced on the finger has to be considered. In most cases it is easy to divide the anterior commissure, but the posterior one, being thicker and indurated, is more difficult to divide. When the opening has been made large enough the finger is passed down within the fan of the chordae tendineae on to each papillary muscle to ensure that there is no cross-union of the chordae. The margin of the fan indicates the limit of the commissure and, in some cases where the mitral ring is small, a complete and satisfactory operation will only leave an opening that will admit one-and-a-half fingers. In other cases where there is a large left auricle and a correspondingly large mitral ring, a full opening would be represented by two-and-a-half fingers. In some instances only one commissure can be divided, particularly when calcification and gross induration complicate the picture.

At the completion of operation, which may involve the entry of the finger several times into the auricle, the control clamp is applied and a double line of non-absorbable sutures used to close the incision. The clamp is then released and the incision inspected for any leak that is larger than a stitch hole which will not bleed for more than a few seconds. In cases where the auricular appendix is small or fibrosed by clot, entrance into the auricle can be achieved by inserting a purse-string suture around a selected area and incising within this zone. The ends of the purse-string suture are held in a Rumel tourniquet, which is a most effective instrument for control when the finger is withdrawn. The pulmonary vein can also be used for entry.

If clot is present, a preliminary wash-back is advisable, and should the finger dislodge any clot during the manipulation this procedure should be repeated, when in many cases clot will be swept out by the high pressure in the auricle. The operation is completed by washing out the pericardium with saline and lightly sewing the pericardial edges prior to closing the chest with a temporary water-seal drain. Before the patient leaves the theatre, particularly if there has been any suggestion of thrombosis, all pulses in the limbs should be checked in case an embolus has occurred. Should an embolus be recognized in a limb, a decision has to be made as to whether sympathetic block and vascular relaxants will suffice or whether the operation of embolectomy should be undertaken.

If a large " saddle " embolus is impacted at the bifurcation of the aorta, this should be removed and a satisfactory circulation re-established in both legs. Cases have occurred in which no damage has been caused by conservative treatment, but this is the exception rather than the rule.

d. Post-Operative Treatment

On return to bed the patient is given full oxygen therapy and sedatives until conscious, and the pulse carefully watched in case of irregularities of rhythm. A certain amount of blood-stained drainage will occur from the pleural drain; and when the lung has fully re-expanded, the tube can be removed. The average patient is kept in bed for three to four days and then gradually allowed to resume normal activities, careful watch being taken of the pulse rate. In a successful case one of the first things that patients note is a sense of freedom in the front of their chest, the presence of the constriction or disability having often been so much part of their symptoms that they have not specially mentioned it.

The handling of convalescence follows that adopted for ordinary chest cases but at a rather slower tempo and, in patients with fibrillation, the dose of digoxin has to be carefully adjusted and a decision made as to whether quinidine therapy should be employed to try to restore normal rhythm.

On leaving hospital patients are warned that, if they have an attack of bronchitis or any shortness of breath, they must go to bed and rest; it is the occasional intercurrent infection that upsets the circulatory balance more easily than anything else. Return to normal may take as long as six months, a steady increase in, for example, doing housework and in walking being assured signs of progress.

If the valvotomy is classified as a good result anatomically, the probability is that the clinical result will also be good. An indifferent or poor anatomical result does not usually lead to a long-term satisfactory result, though there are cases in which the surgeon has been able to do little more than forcibly dilate the valve fractionally and yet allow a sufficient improvement in the blood flow to produce marked alleviation of symptoms for the time being.

e. Complications of Operation

Undue manipulation of the heart may produce abnormal rhythm, and in the early days of mitral surgery considerable emphasis was placed on the use of procaine and similar drugs to reduce cardiac irritability. More recently these drugs have not been found necessary, but if any heart shows marked irritability, it should be given reasonable periods of rest during the actual valvotomy. If the finger obstructs the mitral orifice for more than one or two heart beats there is a marked risk of disturbance; this has always to be borne in mind while the finger is in the auricle.

Technical disasters can be divided into two categories: the one in which the wall of the auricle tears or is ruptured, and the other in which the valve leaves themselves are damaged. In the first instance, bleeding from the auricle itself can usually be controlled even though there may be an alarming moment or so until fingers or clamp can control the fountain of blood. In the case of damage to the valve, the greatest risk is a tear of the aortic cusp resulting in regurgitation which may well prove fatal; if the chordae or papillary muscles are torn, a similar circumstance may result with disastrous consequences.

Embolism. The possibility of dislodging a clot or piece of calcium during manipulation has already been indicated and, if compression on the common carotid arteries is maintained during the actual valvotomy, the risk of cerebral embolism is reduced. The embolus will travel towards the periphery and, if of large size, it is arrested at the bifurcation of the aorta. Clot that has been well organized within the auricle is not likely to be dislodged, the dangerous type being a red granular thrombus that has probably formed within a week or ten days prior to the operation. For this reason complete immobilization in bed of patients with fibrillation before operation may be dangerous. The use of anticoagulants—if there is a history of embolism—is often considered, but, though there are reports to the contrary, their use in the immediate post-operative phase is not advisable.

An interesting syndrome has been noted in a number of cases about the third or fifth post-operative day; this consists of tachycardia associated with distress and a suggestion of cardiac failure. Some authorities feel that this is due to a reactivation of the rheumatic process, but the cause appears to be the result of blood oozing from the suture line in the auricle and accounts for the presence of a blood-stained effusion persisting for some days after the operation.

Disturbances of rhythm during the post-operative phase are fairly common, but, if a heart in regular rhythm goes into fibrillation, a period of a week or ten days should be allowed to elapse before an attempt is made to restore normal rhythm with quinidine.

The first real effect of a successful operation is the sense of freedom within the chest and, before long, patients realize that exertion does not cause them to become short of breath and they also find that they can lie flat. Admittedly, there is always a measure of psychological benefit following operation, but before patients leave hospital they are usually aware of marked physical improvement. Later progress is measured by increasing ability to undertake, for example, household tasks and to walk upstairs or uphill, and at least six months should be allowed to lapse before deciding that the maximum benefit has been obtained.

Pregnancy can hardly be regarded as a complication of the operation but it is a common sequel because patients on returning home have for the first time for months or years been able to indulge in intercourse. The average patient after operation does not tolerate the strain of pregnancy well during the first year or 18 months and has to be warned to that effect. The operation of valvotomy during pregnancy is, however, frequently well sustained and a number of patients operated on in the last few months of pregnancy have undergone normal delivery without distress. This does not imply that every patient with mitral stenosis and pregnancy requires immediate valvotomy; in some cases pregnancy has to be terminated, but in others it is allowed to proceed to term and the heart operation considered later.

3. Results

One of the most satisfactory features of mitral surgery has been the comparatively low mortality. Most surgeons when embarking on this field were prepared to accept a mortality and morbidity rate a good deal higher than has actually been encountered. In my series of 282 cases there were 9 deaths occurring at or within the first few weeks of operation (a figure of just over 3%). Complications which left signs of permanent damage were uncommon and were principally the result of embolism. Cerebral embolism is always alarming and serious, but, with the exception of one patient who

developed a lasting hemiplegia, recovery was satisfactory. Peripheral embolism occurred in five instances, and three of these were " saddle " emboli impacted at the aortic bifurcation. One patient treated conservatively recovered; the others were subjected to immediate embolectomy and one made a satisfactory recovery. Regurgitation as the result of operation caused an adverse clinical picture in only two cases, apart from one patient who died after operation.

In assessing the results, allowance has always to be made for the subjective improvement that invariably follows an operation. In general, results classed as excellent, very good or good, are obtained in 65–70% of the patients. About another 15–20% gain some benefit, but still have symptoms attributable to valvular disease. In this series, 4% succumbed at a variable period following operation, usually from heart failure consequent on bronchitis in patients with damaged heart muscle. The remainder were not helped by operation and indeed some of these patients have only been submitted to intracardiac exploration.

Recurrence of stenosis after operation cannot be ignored and in future years it may become a problem, but at the present time the so-called recurrence cases are often those operated on early in the individual surgeon's experience and in which the valvotomy has not been as complete as in later cases. Pulmonary hypertension does not always fall after valvotomy, and an interval of at least six months should be allowed before taking the final readings, but the clinical course does not necessarily follow the fall in pulmonary artery pressure. There may be a satisfactory early result as regards symptoms, with a minimal change in pressure curves, but in general a long-term good result is accompanied by restoration of the pulmonary artery tension towards normal limits. This is most likely to occur where the hypertension has been of short duration and not associated with myocardial changes.

The success of mitral valvotomy has led to many patients who suffer from rheumatic heart disease requesting operation, and the problem in patients who are not subjects of " pure " mitral stenosis is to decide how much damage has been caused by this narrowing in relation to other valvular defects. Aortic stenosis has recently become amenable to surgical treatment, and a number of patients have had both mitral and aortic stenosis relieved at the same time, but the problem remains principally one of assessing the part played by the various lesions. The period of follow-up will have to be longer before deciding that the improvement produced by valvotomy is lasting, but in patients in whom the rheumatic process has been quiescent at the time of operation there is little evidence of recurrence.

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SURGERY OF THE AORTIC VALVE

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1 Surgical anatomy and pathology
2 Operations for aortic stenosis
3 Operations for aortic incompetence
References

The success which has attended operations for mitral stenosis engendered the hope that the diseased aortic valve might be equally amenable to surgical intervention. This hope has not been entirely fulfilled, and the endeavours to date have been disappointing not only because the operation mortality has been high but because the results of operation have not been clinically good in the majority of patients. All the important references to surgery of the aortic valve can be found in *Surgery of the heart* (Bailey, 1955).

1. Surgical Anatomy and Pathology

The aortic valve is composed of three cusps which balloon out in diastole and prevent regurgitation by apposition of their margins. Accurate approximation of the cusps is attained by the corpora arantii which come together in the middle of the aperture. More than four hundred years ago Leonardo da Vinci showed that the pathway between the aortic cusps was triangular in shape so that its functional size could not be computed by measuring the circumference of the aortic ring, or by passing fingers through the aperture in a dead heart. The accuracy of this conception was recently verified by McMillan (1955) at St Thomas's Hospital. He devised a machine which could photograph the valves of the dead heart working in a stream of water regulated to imitate systole and diastole. This method has been used to show the normal movements of the valves as well as the limitations imposed by disease and the possible results of surgical operations.

Congenital anomalies of the aortic valve are rare but of interest to the surgeon because some lend themselves to operative correction. Several varieties exist. Bicuspid valves may be of no clinical importance, but can cause incompetence when associated with coarctation of the aorta. Bicuspid valves may also be involved in any of the acquired destructive diseases of the heart, and the surgeon who assumes that the valve is tricuspid may rupture a cusp if commissurotomy is attempted. Some children suffering from aortic stenosis have a fibrous diaphragm in place of the normal cusps; in others the actual valve is normal but the outflow tract of the left ventricle is obstructed by a subvalvular obstruction.

In contrast to the mitral, the aortic valve is affected by a variety of acquired pathological conditions other than rheumatic fever. It may, for example, be destroyed by bacterial endocarditis, by syphilis or atherosclerosis. These pathological processes include the ascending aorta from which the coronary arteries arise, and result in hypertrophy of the left ventricle. The combination of low cardiac output, inadequate coronary blood flow and a ventricle which is irritable and holds stitches badly adds to the risks of operation. In many cases the mitral valve is also diseased and the effects of back-pressure are then transmitted to the left atrium and the pulmonary vessels. Another factor unfavourable to surgical interventions is that, on the whole, the patients are older than those suffering from mitral disease.

Aortic stenosis is generally of the calcific type, and the cause is rheumatic fever in most cases. The three commissures become fused together by fibrous tissue and subsequently calcium and bone are deposited, not only obstructing the fairway, but limiting the movement and causing rigidity of the whole apparatus. The majority of valves end by being completely calcified; the cusps no longer move effectively and some incompetence is generally present, as well as stenosis. In most of the specimens examined at autopsy the valve and the adjacent aorta are destroyed beyond hope of operative repair. In these the commissures may sometimes be opened up at post-mortem examination with a knife or strong cutting forceps but, even so, the cusps could never function again (McMillan, 1955). In a typical case of stenosis the left ventricle is greatly hypertrophied but is not necessarily large. In spite of these inauspicious pathological findings there are undoubtedly a number of patients in whom the commissures can be separated by the surgeon and an efficient valve obtained. Bailey (1955) has stated that, in his experience, it is easier to treat the calcific valves than those which are fibrous; but, at the moment, there is no way of picking the favourable from the unfavourable cases except by surgical exploration.

Aortic incompetence may be caused by any type of endocarditis, by trauma, or by surgical correction of aortic stenosis. If the lesion is due to rheumatic fever, the mitral valve is generally affected as well. If syphilis is the cause, destruction of the valve is associated with a special type of arteriosclerosis of the aorta. In this the valve ring dilates, the cusps become thickened and rolled back so that the edges cannot meet. The endocardium below the valve is also affected and the left ventricle dilates (cor bovinum). In some cases the cusps rupture. The signs of aortic incompetence may precede subjective symptoms, but once symptoms develop the outlook is bad.

It has been argued that patients suffering from aortic incompetence are not so dangerously incapacitated as those having aortic stenosis, and that it might be advantageous to convert obstruction to insufficiency by disrupting or removing the valve. Surgeons are of the opinion that merely to perforate a way through the obstruction by stretching the margins of the aperture is undesirable, and clinical success depends upon overcoming the deformity by accurately re-establishing the anatomical commissures and so achieving a functioning valve.

2. Operations for Aortic Stenosis

The earliest attempts to correct aortic stenosis in Great Britain were made from the distal side of the valve. In 1946 Brock of Guy's Hospital, London, devised an instrument which he inserted through the subclavian artery on the right side and passed down to the vicinity of the obstruction. Although he could obtain some fleeting glimpses of the calcified cusps in this way he did not achieve surgical correction of the obstruction. Subsequent endeavours on these lines showed that such an instrument might be passed inadvertently through one of the sinuses of Valsalva and that it was dangerous to attempt procedures of this type. As the techniques for operations upon the mitral valve were improved, surgeons became more familiar with the manoeuvres which were practicable, and many began to attack the problem of aortic stenosis from the left ventricle.

It is correct to emphasize that most of the pioneer work in this field was done by C. P. Bailey of Philadelphia, USA, and his experience exceeds that of other surgeons. The early operations through the left ventricle, in which the abnormal valve was approached from below, were accompanied by a high operation mortality. It was found that haemorrhage from the puncture in the ventricle could not always be controlled; that prolonged or strenuous manoeuvres were quickly followed by ventricular fibrillation; that the valve could seldom be reached and opened by the finger alone and that, in consequence, some form of mechanical dilator was necessary. One by one these difficulties were reduced, and Bailey eventually devised a triradiate dilator whose blades could be made to open in the expected position of the commissures. Using this instrument he reported a number of successful operations and was able to observe great benefit to some patients. The ventricular approach has been used successfully by many surgeons in Great Britain but few have published convincing clinical results. The most encouraging cases have been those reported by Logan of Edinburgh (unpublished communication to the Society of Thoracic Surgeons of Great Britain and Ireland, 1954), who has applied the technique to a series of children suffering from congenital aortic stenosis. Working at Hammersmith Hospital in London, Aird, Melrose, Cleland & Lynn (1954) reported an operation upon a case of aortic stenosis, using an assisted extracorporeal circulation. The underlying idea was to test the possibility of relieving the heart of some of its load in these difficult cases. The operation was done by W. P. Cleland, and was successful.

At a recent meeting of cardiovascular surgeons held in Detroit, USA, Bailey stated that during the last five years he has operated upon 155 patients by the ventricular route; 68 of these were suffering from pure aortic stenosis, and in this group the operation mortality was 28%. In 87 cases there was mitral stenosis, as well as aortic stenosis, and both valves were dealt with at the same time; the mortality in this second series was 18%. At first sight it seems surprising that the double operation should carry a lower mortality than that performed for aortic stenosis alone; but the explanation is that when the mitral valve is obstructed, as well as the aortic, the left ventricle is protected from the effects of excessive filling: it is small, and the problem of primary haemorrhage is not so acute. The late clinical results of these operations have not yet been fully described. At the same meeting W. H. Muller of Charlottesville, USA, reported 37 cases operated upon by the ventricular route; 26 of these had aortic stenosis, 10 had aortic and mitral stenosis, and one had aortic stenosis and coarctation of the aorta. There were 10 operation deaths (27%), and the follow-up showed that 16 patients had attained a good clinical result; in five there was improvement and the remainder were disappointing. Glover has reported (1954) that his over-all mortality has been reduced to 15%.

Being dissatisfied with the mortality of these operations, Bailey has devised a new approach to the aortic valve from the distal side. In essence this consists in sewing a sleeve of pericardium, or other substance, to the ascending aorta just above the valve, and in using this sleeve in the same way as one uses the auricular appendage to enter the left atrium for a mitral operation. Through this operating tunnel the aortic valve can be palpated with the finger directly, and cutting instruments can also be inserted. He reported 61 cases, in 40% of which he split the commissures, using his finger alone: 41 patients had pure aortic stenosis, and in these the operation mortality was 12%; in 20 patients both the aortic and mitral obstructions were dealt with at the same operation, and the operation mortality was 5%. Thus the mortality is much lower than when the operation has been done through the left ventricle.

If these experiences are confirmed it would appear that the retrograde approach to the aortic valve is to be preferred at the present time; but the technical difficulties of sewing on the operating sleeve should not be under-estimated.

S. J. Sarnoff of Bethesda, USA, has in dogs successfully joined the apex of the left ventricle to the descending aorta, thus by-passing the aortic valve completely. The technique has not been applied to human patients, but the dogs are alive and well two years after the by-pass has been introduced.

3. Operations for Aortic Incompetence

The problems presented by aortic incompetence are even more formidable than those of stenosis, and, as yet, no success has been claimed for any direct operations upon the valve itself. Brock (1954) has inserted a flap of pericardium into the aorta above the valve in such a way that, during diastole, it occluded the incompetent orifice. The two patients on whom this operation was performed died. In spite of the technical difficulties, flap valves of various kinds have been introduced in experimental animals by McMillan and others, but they have not proved efficient. It has been shown that the wear and tear upon grafted flaps is such that they disintegrate in a short time.

Bailey experimented with various methods of passing an external constricting suture or tape around the aorta below the origins of the coronary arteries. This method of tightening the dilated aortic annulus is applicable only where the valve cusps are relatively normal, and has not been applied by other surgeons.

Hufnagel of Washington (Hufnagel, Harvey, Rabil & McDermott, 1954) has attacked the problem from another angle. He has devised a plastic ball-valve which he inserts into the descending aorta below the origin of the left sub-clavian artery. The aorta is divided across, and the ball-valve is inserted between the two ends; it is joined to the aorta by specially devised plastic cuffs employing the principle of multiple-point fixation. Admittedly this does not solve the problem of aortic insufficiency but it goes some way to alleviating the strain on the left ventricle. Hufnagel points out that there are many causes of aortic insufficiency and that the patients he has treated have all been dangerously ill. Indeed a number died in the wards whilst they were awaiting surgical treatment, and others had to be taken to the operating-theatre several times before successful induction of anaesthesia could be achieved. Using the plastic valve, Hufnagel has operated upon 80 patients of whom at least 50% were in the terminal stages of heart failure. The hospital mortality of all operations was 20%, and 20% of the survivors have died within two years of leaving the hospital. But the remainder are alive and many are following a useful occupation.

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PROBLEMS IN THE SURGICAL TREATMENT OF VALVULAR INCOMPETENCE

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1 Prognosis of valvular incompetence
2 Measurement of regurgitation
3 Assessment of results of operation for mitral incompetence
4 Procedures for the correction of mitral incompetence
5 Procedures for the correction of aortic incompetence
6 Conclusions
References

Incompetence of the tricuspid valve is most commonly either the result of stretching of the annulus in congestive heart failure or a relatively unimportant defect in multivalvular disease. Incompetence of the pulmonary valve is usually secondary to pulmonary hypertension, the commonest cause of which is mitral stenosis. If the cause of the hypertension is removed, the pulmonary valve becomes competent. Therefore valvular incompetence on the right side of the heart does not call for direct surgical treatment. On the other hand, the serious consequences of mitral and aortic incompetence have stimulated much work, both experimental and clinical, with the aim of diminishing regurgitation through these valves.

Pure valvular incompetence can be produced in the laboratory, but differs from the natural lesion in the absence of primary dilatation of the annulus and sclerosis of cusps or chordae and especially in the suddenness of its production. Experimental valvular incompetence is likely to cause either little impairment of the circulation or early post-operative death, rather than chronic illness. It is of greater value in the study of the dynamics of incompetence than in the design of a palliative operation.

Some of the mechanical principles which are involved in the surgical treatment of aortic and mitral incompetence have been discussed by Brock (1954).

1. Prognosis of Valvular Incompetence

Little interest was taken in the natural history of aortic and mitral incompetence until their surgical treatment was envisaged. The prognosis is affected not only by the severity of the incompetence but also by rheumatic myocarditis, associated valvular lesions, secondary cardiac enlargement and, in the case of aortic incompetence, the extent to which the aorta has been affected by atheroma, syphilis and calcification. Both diseases run a long course, and a reliable prognosis can rarely be made until downward progression has been observed. The selection of patients for operative procedures, the efficacy of which is incompletely established, is restricted to those who are unlikely to survive for long without mechanical help. By the time that stage can be recognized, the heart is considerably enlarged ; it tolerates interference poorly and, because of its mere bulk, is difficult to manipulate at operation.

2. Measurement of Regurgitation

The effect of aortic and mitral incompetence can be assessed by the degree of left ventricular enlargement and hypertrophy as detected by clinical, radiological and electrocardiographic methods, but, in spite of a number of attempts, there is no accurate means of measuring blood flow through the incompetent valve. In mitral incompetence, palpation of the regurgitant jet at cardiotomy gives the best impression of the amount of regurgitation. The impression is influenced by many factors, and its description tends to acquire an appearance of mathematical accuracy. Its value is least when it is most required, that is, in the comparison of the regurgitation immediately before with that immediately after an operation on the mitral valve. The opportunity for digital palpation of the jet does not usually arise in the es nation of aortic regurgitation.

3. Assessment of Results of Operation for Mitral Incompetence

The frequency with which mitral incompetence accompanies mitral stenosis or is produced by valvulotomy for stenosis has led a few surgeons to attempt correction of the incompetence at the same time as the stenosis. This work is done in unrewarding circumstances. It is begun in ignorance of the amount of mitral incompetence that will significantly damage the circulation and is ended with little chance of recognizing what has been achieved. Abolition of all signs of incompetence would indicate correction of the incompetence, but that is an unlikely result of any procedure now in use. Alteration in persisting signs of incompetence could not confidently be ascribed to diminution of incompetence. After operation in a case of pure mitral incompetence, a reduction in the signs might reasonably be interpreted as showing diminution of incompetence, due allowance having been made for the improvement in well-being and exercise tolerance which commonly lasts three to nine months after simple cardiotomy in mitral disease. However, pure mitral incompetence is rare (Brigden & Leatham, 1953).

4. Procedures for the Correction of Mitral Incompetence

Four types of procedure have been employed : (i) constriction of the annulus ; (ii) fixation of a structure in the cavity of the heart to fill a defect in approximation of the cusps ; (iii) suture of the cusps ; (iv) mobilization or replacement of the cusps.

i. In the study of mitral stenosis, a number of workers used a circular suture at the annulus, to constrict the mitral valve (Ellison, Major, Pickering & Hamilton, 1952). Davila and his associates (Davila, Mattson, O'Neill & Glover, 1954), after a careful study of the anatomy of the annulus, proposed the application of this method to the correction of mitral incompetence. The suture may be difficult to introduce without endocardial damage in the living heart. A ring of endocardial fibrosis related to the suture has been experimentally observed. The method is still unproved, but seems worthy of trial in cases of pure mitral incompetence and of incompetence after valvulotomy for stenosis in which the cusps are mobile.

ii. Harken (Harken, Black, Ellis & Dexter, 1954), following his observation that regurgitation through the mitral valve could be diminished or abolished by placing the finger in the defect between the cusps, has made a sustained effort to correct regurgitation by the use of a Lucite prosthesis which he calls a

baffle. He has operated on patients with pure mitral incompetence and can reasonably claim that improvement observed depends upon reduction of regurgitation. His progressive modification of the procedure indicates a lack of complete satisfaction with it. The prosthesis now used is spindle-shaped and of a size calculated from the effect of the finger in the incompetent valve. It traverses the mitral orifice at the site of maximum reflux and is fixed by suture to the heart wall below the anterior commissure and above the posterior. The natural mobility of the cusps is preserved, and the prosthesis has no moving parts and is unlikely to wear out.

To produce a similar effect, the left auricle has been invaginated through the mitral valve into the ventricle and fixed by suture to the ventricular wall (Davila *et al.* 1954). This has the theoretical advantage that none but an endocardial surface is exposed in the blood stream, but it would seem that the size and position of the auricle are less readily adjustable to the requirements of the mitral valve.

Bailey (Bailey, Lacy & Harris, 1951) has passed pericardial tubes, free and pedicled, through the ventricle below the mitral valve, so that during ventricular systole they may fill the defect left by the damaged cusps. Being a living tissue, pericardium does not necessarily retain its original size and shape, and clot sometimes forms upon the surface presenting towards the auricle, causing progressive valvular obstruction. A cylinder or spindle of polyvinyl sponge, pedicled on the anterior and posterior papillary muscles, has been used in the same way in the laboratory by Blalock (Johns & Blalock, 1954), who found that it retained its mobility and did not form clot.

iii. Bailey and his associates (Bailey, Jamison, Bakst, Bolton, Nichols & Gemeinhardt, 1954) have also designed ingenious methods of suturing the cusps of the mitral valve, fixing them together during both systole and diastole, and so diminishing regurgitation at the calculated cost of creating some stenosis.

iv. In some cases, incompetence may be caused or increased by adhesion of the cusps, chordae or papillary muscles to the ventricular wall, and in these Dodrill (1954) has practised digital mobilization of the cusps.

Templeton & Gibbon (1949) attempted excision and replacement of the posterior mitral cusp during temporary arrest of the circulation. This procedure has not been used therapeutically.

5. Procedures for the Correction of Aortic Incompetence

Three varieties of corrective procedure have been devised for aortic incompetence: (i) constriction of the annulus; (ii) addition of artificial cusps or pericardial strips to the valve; (iii) insertion of a homologous or artificial valve distal to the damaged valve.

i. The use of a circumferential ligature, as in the procedure of Davila *et al.* (1954) for mitral incompetence, although it has similar anatomical difficulties, may be of some value if the ring is dilated and the cusps are reasonably sound, a combination of circumstances at least as infrequent in aortic as in mitral disease.

ii. Supplementary cusps in the form of strips of pericardium or other material crossing the aortic lumen above the valve carry the risk of obstruction of the coronary ostia. It is unlikely that any of them could maintain a normal aortic diastolic pressure without the support of a stenosed valve from below. Litwak and his co-workers (Litwak, Gadboys, Scott & Ferrara, 1952) described a method of excising and replacing an aortic cusp in the experimental animal without interruption of the circulation. A diseased valve and hypertrophied left ventricle would be serious obstacles to its use in man.

iii. Hufnagel, after abandoning the use of homologous aortic or pulmonary valves, has designed an efficient and durable Lucite ball-valve and an ingenious method of fixing it in the aorta. Placed just beyond the origin of the left subclavian artery, the valve prevents 75% of the regurgitation, restores the normal quality of the femoral pulses and permits some reduction of cardiac size (Hufnagel, Harvey, Rabil & McDermott, 1954). It is obvious that the fewer aortic branches there are proximal to the artificial valve the greater will be the effect of the valve on regurgitation. The insertion of the valve into the ascending aorta would, however, require a larger operation with either temporary interruption of the cerebral circulation or its maintenance by a by-pass, and is therefore inapplicable in a patient with gross cardiac damage. In addition, a valve proximal to the innominate artery might impair the diastolic filling of the coronary arteries as a consequence of the sudden fall of pressure in the column of blood between the incompetent aortic valve and the artificial valve close above it.

For some unknown cause, the Hufnagel operation is often followed by a fairly severe anaemia during the early post-operative weeks.

6. Conclusions

With the exception of Hufnagel's operation for aortic incompetence, it is doubtful that any of these procedures which have been applied in man provide even a temporarily satisfactory method of treating valvular incompetence. There is no evidence to justify their widespread clinical trial, and little for their extension beyond the research or surgical units in which they have been developed. Information on the construction of valves and the behaviour of foreign materials in the blood stream grows steadily. Increasing knowledge of the use of hypothermia[1] to lengthen the safe time of arrest of the circulation, and of techniques for by-passing the left side of the heart, may finally permit the resection and replacement of the mitral cusps and possibly also of the aortic cusps, so making severe damage of the cusps, whether causing stenosis or incompetence, a single surgical problem.

[1] See papers by Delorme (p. 221) and Ross (p. 226) in this number of the Bulletin.—Ed.

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ARTERIAL RECONSTRUCTION

With Special Reference to the Use of Homografts

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1 Direct suture
2 Thrombo-endarterectomy
3 Autogenous vein grafts
4 Homologous arterial transplants
  a Sterilization of contaminated blood vessels
  b Fate of a blood-vessel transplant
5 A prosthesis of foreign material
6 Indications for arterial reconstruction
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7 Results
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The flow through an artery may be restored by a direct anastomosis, an embolectomy or thrombo-endarterectomy, an autogenous vein graft, a homologous arterial transplant or by a prosthesis of plastic cloth.

1. Direct Suture

Direct suture is the ideal method of repairing an injury, and it is surprising how often it is possible to close the gap by direct suture after a diseased segment of an artery or vein has been resected. This is particularly so in patients with arteriosclerosis; an arteriosclerotic artery is often lengthened and tortuous; this, after mobilization, allows the surgeon to close a fairly long defect by direct suture.

During the past four years we have repaired defects in the arteries of four elderly patients in this way. The first patient was aged 74; during the course of an operation for carcinoma of the mouth, a mass of lymph nodes was found to be adherent to the common carotid artery; 3.5 cm. of this vessel were resected and after mobilization a direct end-to-end anastomosis performed; the patient is alive and well four years later. In this case the anastomosis was performed under only slight tension in spite of the fact that 3.5 cm. of the common carotid artery had been removed; the previous tortuosity of the artery permitted this. The second and third patients were both over 75: each ruptured a brachial artery as part of a fracture dislocation of the shoulder joint, and the gap resulting was closed by a direct end-to-end anastomosis. The fourth patient was of particular interest: she was aged 66 and had a localized partial occlusion of the left common and internal carotid arteries; this produced intermittent attacks of hemiplegia and blindness of the opposite eye; resection of the diseased segment of artery 3 cm. long, and reconstruction by a direct anastomosis, has prevented further attacks of paralysis or blindness (Eastcott, Pickering & Rob, 1954).

2. Thrombo-Endarterectomy

The first attempts to remove the thrombus from an occluded arteriosclerotic artery were made by dos Santos, who in 1947 described the operation of thrombo-endarterectomy. This operation was later modified by Reboul (Reboul & Laubry, 1950), and several surgeons, including Leriche & Kunlin (1947), Fontaine & Hubinot (1950), Freeman & Gilfillan (1952) and Forty (1952), have reported their experiences. The report of Reboul & Laubry (1950) states that, in 93 operations, the results were as follows: 44 arteries (47%) remained patent, in 38 (41%) clots formed again, and 11 patients (12%) died. In our hands the results have been disappointing and approximate to those reported by Reboul and Laubry. Nevertheless we have not entirely abandoned the operation, and consider that an occasional case is suitable.

The operation of thrombo-endarterectomy was the first direct attack upon arteriosclerosis and consists of enucleating the thrombus through a plane of cleavage in the media, after the vessel has been opened through a longitudinal incision. We find that the main disadvantages have been the high recurrence rate and the necessity for a long incision in all but well-localized lesions. We now reserve it for very small localized occlusions of large vessels, such as the iliac arteries; to show how infrequent it is to find a suitable case, we have performed this operation only four times since 1951.

An ideal case is illustrated in Plate VII, figs. A, B. This patient was aged 48; 10 years earlier he began to get intermittent claudication in his right leg after walking 1 or 1½ miles[1]; this gradually got worse and was not improved by a lumbar ganglionectomy. Just before he consulted us his walking distance was reduced to 30 yards[2] and he was unable to work. On examination he had a weak right femoral pulse with no pulsation palpable in the right limb distal to this point. A plain radiograph showed a densely calcified nodule in the region of the right common iliac artery. An arteriogram (Plate VII, fig. A) demonstrated an irregular filling defect more than 1 cm. in length, occupying most of the lumen of the right common iliac artery. At operation this defect was found to be due to a calcified and almost pedunculated plaque which largely obstructed the lumen of the artery, with macroscopically normal vessels above and below. This plaque was removed. Full pulses returned to the dorsalis pedis and posterior tibial arteries. The patient has resumed work as a general labourer. Plate VII, fig. B, is an arteriogram taken one month after the operation; it shows a satisfactory flow through the right common iliac artery. The results in two of our other three patients are good; one, a naval officer aged 47, is still on active duty four years after his operation, but the last of our four patients treated since 1951 thrombosed his aortic bifurcation again about one month after the operation, and his limbs are now worse than they were before operation.

We therefore reserve the operation of thrombo-endarterectomy for exceptionally well-localized occlusions of large arteries. It is not a good procedure for a vessel of the size of the superficial femoral artery or for smaller ones.

3. Autogenous Vein Grafts

This procedure has many advantages when compared with the alternative methods of bridging the defect with a homologous arterial or venous transplant or a prosthesis of plastic cloth. Unfortunately many of these advantages which have appeared so sound in theory have not been confirmed in practice, particularly as regards the use of autogenous vein grafts to bridge gaps in arteries.

The advantages of using a segment of one of the patient's own veins include: the fact that a blood-vessel bank is

[1] 1 mile = 1.6 km.—Ed.
[2] 1 yard = 0.9 m.—Ed.

unnecessary (this means a great saving of time and money); that the long saphenous or external jugular veins can be sacrificed without inconvenience to the patient (of these the long saphenous is the best because it has a thicker wall and the anastomosis can be performed more easily); that the vein, if it is autogenous, survives transplantation and forms a permanent living union with the host, whilst a homologous arterial transplant merely acts as an inert tube; and lastly, in war, with its attendant difficulties of supply and transport, an autogenous vein graft can be obtained with ease. The reasons why an autogenous vein graft is less satisfactory in practice than in theory include: the fact that in our hands the success rate has been lower when the results of bridging defects in arteries with autogenous vein grafts have been compared with those obtained in similar patients with homologous arterial transplants; that the operation of inserting an autogenous vein graft into an arterial defect is technically more difficult than the insertion of a homologous arterial transplant, largely because of the need for adequate tension if undue tortuosity is not to follow the removal of the clamps and the passage of blood at arterial pressure through the segment of vein. In our view it is this fact, that veins are more difficult to insert than arteries, which accounts for the higher rate of failure, either immediate or late. Moreover, veins inserted into large arteries in the body cavities rupture in a high proportion of cases, because for a vein graft, inserted into an arterial defect, to succeed it is important that the vein should be supported by the tissues of the host. This occurs in a limb where the muscles support the graft, but not in a body cavity. In many patients the saphenous vein is not suitable for use as a graft.

The first successful vein-to-artery autograft in a patient was performed by Lexer and reported in 1912 (Lexer, 1912). By 1913 he was able to report that he had resected three aneurysms of large peripheral arteries and restored continuity by an autogenous vein graft in each case (Lexer, 1913). Two patients were alive and well with patent grafts; one died six days after the operation, but the graft was found to be patent and intact. In Britain, Pringle in 1913 recorded two cases of aneurysm treated by this method (Pringle, 1913); one patient worked for over three years after the operation and, when he died from heart disease, examination of the graft showed it to be patent; the other case of a brachial aneurysm was also satisfactory, and the patient resumed full work as a blacksmith. In these years just before the First World War several other surgeons performed the operation of autogenous vein grafting; among these was Coenen, who in 1913 was the first to use this method of reconstruction after the resection of an arteriovenous fistula (Coenen, 1913).

It was hoped that this operation would prove to be of value in war, and many attempts were made in the Balkan war of 1912 and the World Wars of 1914–18 and 1939–45 to repair injured arteries in this way. The results were unsatisfactory, because of wound infections. In Korea, as reported by Jahnke & Seeley (1953) and by Hughes (1954), this problem was largely solved, and 15 autogenous vein grafts were inserted.

Many surgeons have used autogenous vein grafts in the treatment of arteriosclerotic thromboses of peripheral arteries, and the results have been better than with the operation of thrombo-endarterectomy. Fontaine, Buck, Riveaux, Kim & Hubinot (1951) report that a follow-up showed a patent vessel in 10 out of 28 reconstructions by resection of the thrombosed segment and the insertion of an autogenous vein graft, and in only three out of 27 patients in a similar group treated by thrombo-endarterectomy. These figures, although they show an improvement on previous methods, are still unsatisfactory. As already stated, we prefer homologous arterial transplants for such lesions, and our results will be given later in this paper.

4. Homologous Arterial Transplants

An artery bank is essential if homologous arterial transplants are to be used. The first bank was established by Carrel (1908), who laid down certain principles in his experimental work which have been of value to all other workers in this field. Today arteries may be preserved by refrigeration (Gross, Bill & Peirce, 1949), frozen (Eastcott & Hufnagel, 1951), freeze-dried (Marrangoni & Cecchini, 1951), or preserved chemically (Levin & Larkin, 1909). Of these methods we prefer freeze-drying because of its convenience and because the arteries when used in patients have given results which at least equal those of the other methods. In 1954 we described a simplified technique for freeze-drying arteries (Eastcott, Holt, Peacock & Rob, 1954), and further details will be found in that paper.

a. Sterilization of Contaminated Blood Vessels

There are three ways in which contaminated blood vessels have been sterilized and rendered fit for use in patients. The first is by chemical means (Levin & Larkin, 1909), but this denatures the proteins. The second is by cathode and *x* irradiation (Meeker & Gross, 1951), which can sterilize heavily contaminated material; experimentally such transplants have given satisfactory early results, but calcification develops in many cases within a few months (Hui, Keifer, Deterling, Parshley, Humphreys & Glenn, 1952). The third and best method is with liquid ethylene oxide (Hufnagel, Rabil & Reid, 1954); this technique makes the collection of blood vessels extremely simple. Without any special precautions the arteries are removed from the donor and placed in liquid ethylene oxide; after 20–30 min. they are removed and banked by freezing or freeze-drying. Transplants sterilized in this way have given good early results in clinical practice. But there are certain difficulties about the method: one is that the liquid is explosive and dangerous, and another is that ethylene oxide slightly denatures the proteins of a blood-vessel transplant. In our view these difficulties, particularly the latter, make it wiser to take the extra trouble and use aseptic methods when collecting blood vessels, rather than an antiseptic technique.

b. Fate of a Blood-Vessel Transplant

An autogenous graft of artery or vein survives after transplantation and forms a permanent living union with the host. An autogenous artery graft months or years afterwards is indistinguishable both microscopically and macroscopically from a normal artery. On the other hand, homologous arterial transplants merely function as inert tubes; they do not form a living union with the host, yet in spite of this they work well.

The standard teaching is that a homologous arterial transplant is kept open by the force of the blood flow and that over a period of weeks or months the tissues of the host grow in to replace the donor artery. Intima grows out from the ends of the host vessel to provide a new lining for the transplant, and fibrous tissue grows in to replace the media and adventitia. Eventually all that remains of the transplant are the elastic fibres, and these extracellular structures persist for years in

an apparently unchanged state. We now think that this does not happen in man. Recently we have had the opportunity of examining four human arterial transplants which were patent and intact nine, nine, four and two months respectively after their insertion. Three of these were femoral and popliteal transplants 6 cm., 17 cm. and 12 cm. long, and one was an aortic transplant 11 cm. long. All were longer than the usual experimental arterial transplants which have formed the basis of former studies, including our own. These transplants show only a little replacement by the tissues of the host. After nine months the endothelium of the intima has grown out 1–2 cm. from the end of the host artery; in the gap between this true endothelium the transplant is covered by fibrin, collagen and flattened fibroblasts, and the media and adventitia have died; but most of these layers have persisted as dead donor tissue with only a little replacement by the fibrous tissue of the host. The elastic fibres remain. This means that a human arterial transplant more than 4 cm. long consists over its central portion of a layer of fibrin and collagen, two layers of dead and almost amorphous donor artery, the elastic laminae, and some capillary tufts and fibrous tissue where the host tissues have grown in. The persistence of the elastic fibres is probably the main reason why homologous arterial transplants have proved so successful in clinical practice.

5. A Prosthesis of Foreign Material

As already stated, homologous blood-vessel transplants function only as inert tubes; they do not live in the host. This means that any suitable material should serve as well. The search for such a material has been going on for more than 40 years, but it has only been since the end of the Second World War that real progress has been made. Two types of prosthesis have been used, one a rigid tube, notable examples being the glass tube designed by Tuffier and used during the war of 1914–18, and the vein-lined vitellium tube of Blakemore, Lord & Stefko (1942); but the results were poor, and the majority thrombosed. More recently, solid plastic tubes have been used, but they have the disadvantage that clots form at the point of union between the tube and the host artery; these may lead to either complete occlusion or sometimes peripheral emboli. Today the only use for such a rigid prosthesis is to carry a valve, as recommended by Hufnagel, Harvey, Rabil & McDermott (1954) for the treatment of aortic incompetence, but even in this situation clots may form and produce peripheral emboli.

On the other hand, it is probable that prostheses of plastic cloth will eventually displace arterial transplants for the reconstruction of the aorta; for smaller vessels the results are still inferior to those obtained with blood-vessel transplants. Voorhers, Jaretzki & Blakemore (1952) have reported good results with vinyon cloth, and more recently several authors have found various other plastic cloths to be adequate for this purpose. The cloth is either woven into a tube of the appropriate size or bought as a sheet and sewn with a double seam into a tube; it is then sutured into position in the same way as an arterial graft. The plastic cloth must be inert in the tissues, strong, easily sewn without any tendency to split or ladder, it should be slightly porous so that the blood can sweat through without an actual leak occurring, and it should be easily sterilized. Many cloths meet these requirements. So far we have found orlon, an acrylic fibre, to be the best for the purpose. Plate VII, fig. C, is an arteriogram showing an aneurysm of the abdominal aorta which had been caused by tuberculosis of the abdominal lymph nodes eroding the aortic wall; it was excised together with a large tuberculous abscess, and continuity of the aorta restored with a prosthesis of orlon cloth. Plate VII, fig. D, shows the cloth in position; this patient has resumed work as a business executive (Rob & Eastcott, 1955).

6. Indications for Arterial Reconstruction

We believe that these are increasing in frequency and that, as surgery advances, more and more direct vascular surgery will be performed. The following list subdivides the indications for these operations into a number of groups: (i) congenital abnormalities, e.g. a long coarctation; (ii) aneurysms and arteriovenous fistulas; (iii) wounds, first as a primary measure and second if symptoms develop after ligation; (iv) primary thrombosis, e.g. of the popliteal artery; (v) after some emboli; (vi) occasionally in malignant disease; (vii) in some patients with arteriosclerosis.

a. Congenital Abnormalities

The first homologous arterial transplants were used by R. E. Gross for the correction of coarctation of the aorta when the operation of direct anastomosis was not possible. Whilst the majority of patients with coarctation of the aorta of sufficient severity to need operation can have their abnormality corrected without a blood-vessel transplant, it is of great value to know that a transplant is available should it be needed. From our bank nine arterial transplants have been used for bridging the gap after excision of the abnormal segment of aorta in patients of this type; all are well, and the first was operated on four-and-a-half years ago.

The usual reason why an arterial transplant is needed is the presence of an associated aneurysm, and in eight of our nine patients this was the case; in the other patient the constricted segment was so long that a direct anastomosis was impossible.

b. Aneurysms and Arteriovenous Fistulae

If an arterial aneurysm threatens life or is causing sufficient symptoms to merit operation, it is better to restore the blood flow after its removal than to interrupt the main stream with a ligature. In the case of an arteriovenous fistula, particularly if it has been present for some months, the collateral circulation will be so adequate that reconstruction is rarely essential, but when practical it should be performed because, with ligation, symptoms of ischaemia may develop, although gangrene is rare. In all, we have had experience of 39 homologous arterial transplants in patients with aneurysms and arteriovenous fistulae. Twenty-nine left hospital with patent vessels, four thrombosed and six are dead, including three who had had emergency operations for leaking aortic aneurysms.

c. Wounds

The repair of arterial wounds by direct suture has already been described. When this is impossible a blood-vessel transplant should be used. As reported by the USA army surgeons in Korea (Jahnke & Seeley, 1953; Hughes, 1954), 15 autogenous venous and four homologous arterial transplants were used for this purpose. In civilian practice such injuries occur, and a proportion require a blood-vessel transplant. We have repaired seven such injured blood vessels: five were successful, and one thrombosed on the sixth and one on the eighth postoperative day because of wound infections associated with their original injury.

d. Primary Thrombosis (Including Traumatic Thrombosis)

This may be defined as thrombosis of an artery which was previously normal, or nearly so, in a patient who shows little evidence of generalized arterial disease and whose subsequent history shows no further tendency towards a similar episode in another vessel. The important thing is to distinguish between this lesion and the very much more common thrombosis that occurs in patients with arteriosclerosis. Primary thrombosis is a rare lesion.

We have seen nine such cases in patients: seven were thromboses of the popliteal artery, one involved the brachial artery, and the other followed a closed injury to the femoral artery. All have been treated with a blood-vessel transplant; it must be stressed that we see an unusually large number of these cases because the patients are referred to us for a reconstructive operation. The first patient was operated on over four-and-a-half years ago; a homologous arterial transplant was inserted into the popliteal artery; he is alive and well today, with a patent vessel. Of the remaining eight patients, six left hospital with patent vessels, in one the brachial thrombosed again immediately after the operation, and the other developed a wound infection which resulted in a secondary haemorrhage four weeks later. Of the seven who left hospital with patent vessels, one was readmitted nine months later with ascites due to cirrhosis of the liver; while this patient was in hospital the vessel thrombosed.

e. Arteriosclerosis

This general disease affects the arteries of the whole body. In some an aneurysm results, but in many patients the symptoms are caused by a localized occlusion of a major artery. In a few of these patients a blood-vessel reconstruction is justified, particularly if a large vessel such as the aorta or common iliac artery is involved.

7. Results

To date we have repaired 104 arteries with a transplant or plastic cloth prosthesis. Eighty-one of the patients left hospital with patent vessels, 14 developed thrombosis in hospital, one had to be ligated because of a secondary haemorrhage, eight died, and nine had operations requiring hypothermia.

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ARTERIAL RECONSTRUCTION

(FIGS. A—D)

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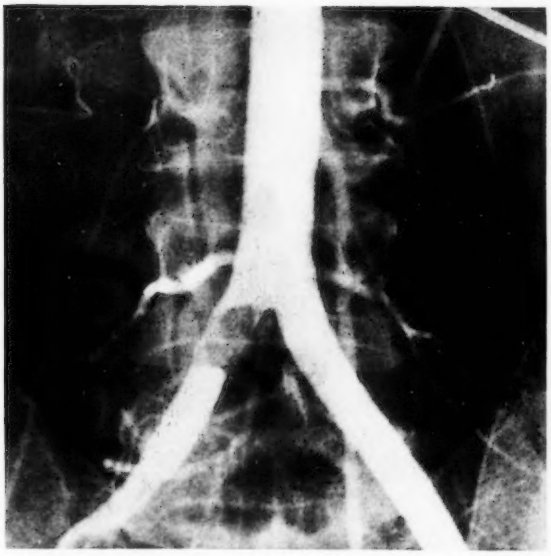

FIG. A. Arteriogram of a patient aged 48 who developed intermittent claudication in the right leg after walking an average distance of about 50 yards. Note the small and localized obstruction to the right common iliac artery

FIG. B. Arteriogram of the patient shown in fig. A, after thromboendarterectomy. He can now do a full day's work as a general labourer

FIG. C. Arteriogram showing an aneurysm of the abdominal aorta, which was due to erosion of the aortic wall by tuberculous granulation tissue from a cold abscess arising in the abdominal lymph nodes

FIG. D. Post-operative arteriogram showing a prosthesis of orlon cloth bridging the gap in the aorta, which resulted from excision of the aneurysm shown in fig. C

PLATE VIII (FIGS. A, B)

HYPOTHERMIA

(FIG. A)

E. J. Delorme

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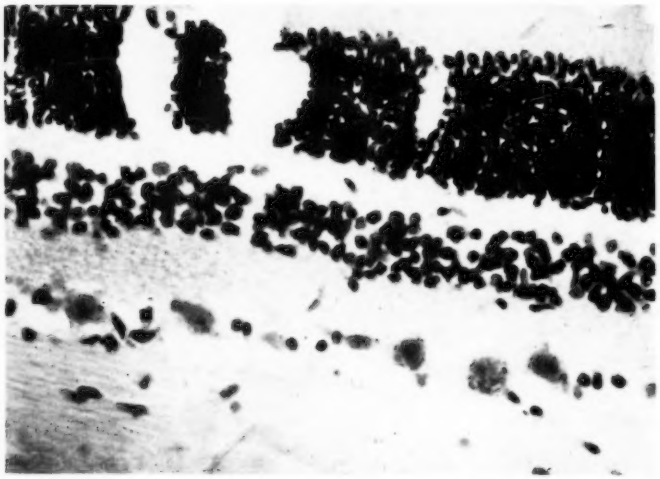

FIG. A. Photomicrograph showing absence of chrom atolysis in ganglion cells of a dog's retina, after on hour of cerebral ischaemia

(magnification × 400

PRACTICAL APPLICATIONS OF HYPOTHERMIA

(FIG. B)

D. N. Ross

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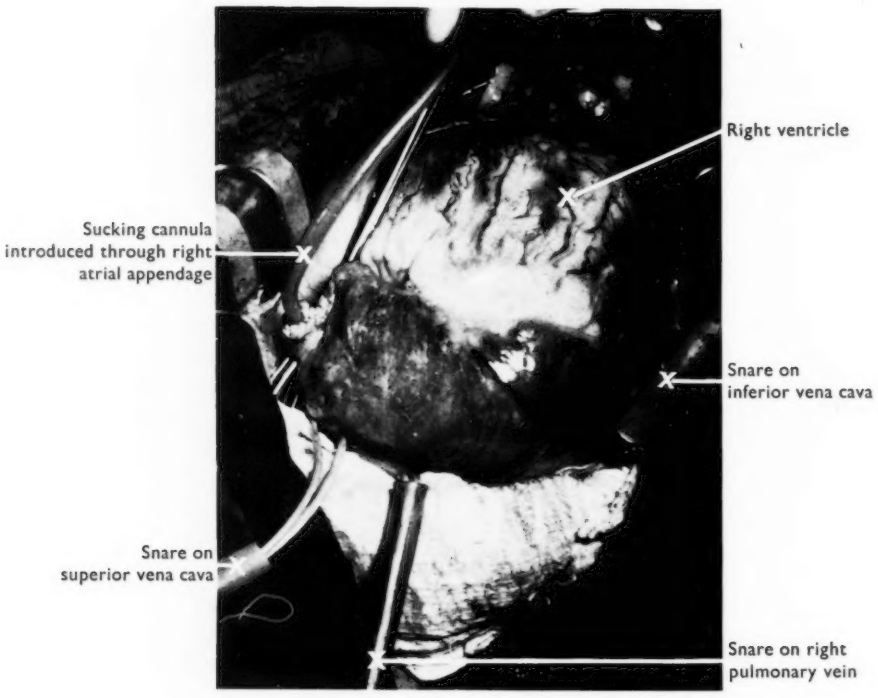
Right ventricle
Sucking cannula introduced through right atrial appendage
Snare on inferior vena cava
Snare on superior vena cava
Snare on right pulmonary vein

FIG. B. Human heart exposed at operation

HYPOTHERMIA

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1 Sources of information
2 Methods of inducing hypothermia
3 Conclusions based on experiment
a Beneficial effects of cooling
b Potential dangers of cooling
4 Theoretical considerations
References

The term " hypothermia " is here used to denote an induced or spontaneous reduction of total body temperature. Confusion in terminology can impede the interchange of precise information in a new field and it should be noted that hypothermia is not synonymous with any form of natural hibernation or with local cooling, nor are the loose terms " refrigeration anaesthesia " or " freezing technique " suitable in this connexion.

The rationale for the reduction of tissue temperature has now been demonstrated both in the laboratory and in clinical application. It lies essentially in the reduction in oxygen requirements coincident with the depression of cellular metabolism by cold. This lowered respiratory activity of the cell prolongs its survival in all circumstances in which cell vitality is endangered by defective oxygen distribution. In addition to this oxygen-sparing mechanism there is a thermal depression of harmful enzyme activity. Cooling not only delays the onset of anoxia during failure of oxygen supply but postpones the secondary enzymic changes that ordinarily follow upon anoxic injury, and that lead ultimately to the death of the cell.

1. Sources of Information

Only in the past two decades has the physical agency of cold in the form of a reduction in total body temperature been applied as a therapeutic measure. Although its present therapeutic indications are distinctly limited by lack of knowledge of the exact physiological and biochemical alterations involved, the principle is a broad one and one that has excited interest among research groups in many countries.

Before 1938, when Temple Fay in the USA first described his attempts to control malignant disease by hypothermia brought about by surface cold (Fay & Henry, 1938), information on the subject was restricted to observations on accidental hypothermia in man (Reincke, 1875 ; Nicolaysen, 1875) and sporadic studies on experimental hypothermia in animals (Walther, 1865; Tait, 1922). The work of Fay, although failing in its immediate intent, fortunately stimulated further investigation into the physiology of hypothermia both in man (Dill & Forbes, 1941; Smith, 1942; Talbott, 1941) and in experimental animals (Barbour, McKay & Griffith, 1943; Penrod & Hegnauer, 1948). Investigations into climatic effects of cold, in the post-war period, have made significant contributions to our knowledge, particularly in regard to the endocrine responses to cold (Wilson, 1953; Josiah Macy, Jr Foundation, 1952, 1954).

Hypothermia as an aid to cardiac surgery developed directly from the work of Bigelow, Lindsay, Harrison, Gordon & Greenwood (1950). Bigelow's fundamental observations that the total oxygen consumption of the warm-blooded animal could be reversibly lowered by hypothermia without incurring an oxygen debt led to subsequent experiments in which he excluded the blood from the heart chambers for 15 min., with recovery of the animal. Open surgery of the heart, an objective not hitherto attained by other means, became at once a clinical possibility, and surgeons were not long in putting this principle to the test (Cookson, Neptune & Bailey, 1952; Lewis & Taufic, 1953; Swan, Zeavin, Holmes & Montgomery, 1953; Swan & Zeavin, 1954; Dundee, Gray, Mesham & Scott, 1953; Bailey, Cookson, Downing & Neptune, 1954).

Bigelow further recognized, as did Dill and Forbes, the need for deep anaesthesia in order to depress reflex shivering and muscle tremors which otherwise cause a rise in oxygen expenditure, with exhaustion and a prohibitive mortality. The tendency to venous engorgement during cooling and its relation to cardiac embarrassment were also noted by Bigelow and his co-workers. Many technical details (including the electrical pace-maker and radio-frequency re-warming) were first proposed and tested by this group (Callaghan & Bigelow, 1951 ; Bigelow, Hopps & Callaghan, 1952).

In 1949 there was begun in Edinburgh an experimental study on the protective effects of hypothermia in various types of tissue ischaemia and shock (Delorme, 1952). It was considered that the conventional method of extracting heat from the body surface was not the ideal method of lowering body temperature and that this could be more safely achieved by cooling the body uniformly by way of the blood stream. While this work was in progress another method of blood-stream cooling was described by Boerema, Wildschut, Schmidt & Broekhysen (1951), and attention was drawn to its advantages. Ross later adopted the Edinburgh method of pervascular cooling and he gives a full description of the present technique to which he has added a number of refinements (Ross, 1954).[1]

2. Methods of Inducing Hypothermia

In the past three years, three different techniques for procuring hypothermia have been used experimentally and each has been subjected to limited clinical trial. The first is a method depending primarily upon the use of depressant and so-called sympatheticolytic drugs, and secondarily upon moderate surface cooling, as introduced in France by Laborit & Hugenard (1951). The second method, derived directly from the early work of Temple Fay, depends primarily upon intensive surface cooling and to an almost equal degree upon heavy sedation and anaesthesia. This is the method which has been used by Bigelow and by the majority of later investigators. The third method depends primarily upon blood-stream cooling and requires only minimal anaesthesia and no drugs (Boerema *et al.* 1951; Delorme, 1952; Ross, 1954).

It is not possible to foresee which of the methods will come into general use, but it is probable that the drug method will

[1] See also Ross, p. 226 of this number of the Bulletin.—Ed.

prove inadequate for surgery involving any degree of circulatory interference because it has not been shown to be possible to reduce body temperature and oxygen consumption significantly in man or larger mammals by chemical means, although it has been possible to do so in small animals. However, it is hoped that new drugs will play an important part both as accelerators in the cooling procedure and as inhibitors of cardiac arrhythmias. In surface cooling, the "cold stress" responses and calorigenic effects of skin stimulation are deleterious and must be minimized by deep anaesthesia or drugs. Interference with hepatic inactivation and renal excretion of these drugs by cold may render anaesthetic control difficult and resuscitation from the cooled state precarious.

Temperature levels may be reached in the periphery during surface cooling, which are capable of producing delayed lesions in muscle and nerve tissue in man after even relatively short periods of exposure (Denny-Brown, Adams, Brenner & Doherty, 1945). Fat necrosis has been described following surface cooling in infants (Collins, Stahlman & Scott, 1953). On the other hand, Andjus & Smith (1954) have reported full recovery in rats from body temperatures approaching zero centigrade. The final criterion must be the safety of the method as applied to man.

A common purpose may be discerned in all three methods: the safe reduction of oxygen requirements with, at the same time, control or inhibition of stress responses. The drug method is concerned chiefly with the second of these factors. In the author's experience, pervascular cooling gives a higher degree of protection in ischaemic states with a lower degree of risk than do the other methods. This is believed to be due to the greatly reduced stimulatory effect, and to the smoothness and uniformity of cooling. The effective stimulus for shivering has been shown to be predominantly somatic in origin (Jung, Doupe & Carmichael, 1937; Keller, 1938). In the case of infants and young children, however, surface cooling may be both effective and safe because of the presence in early life of less vigorous hypothalamic responses to surface cold and a greater surface area in relation to body mass resulting in a more efficient heat loss.

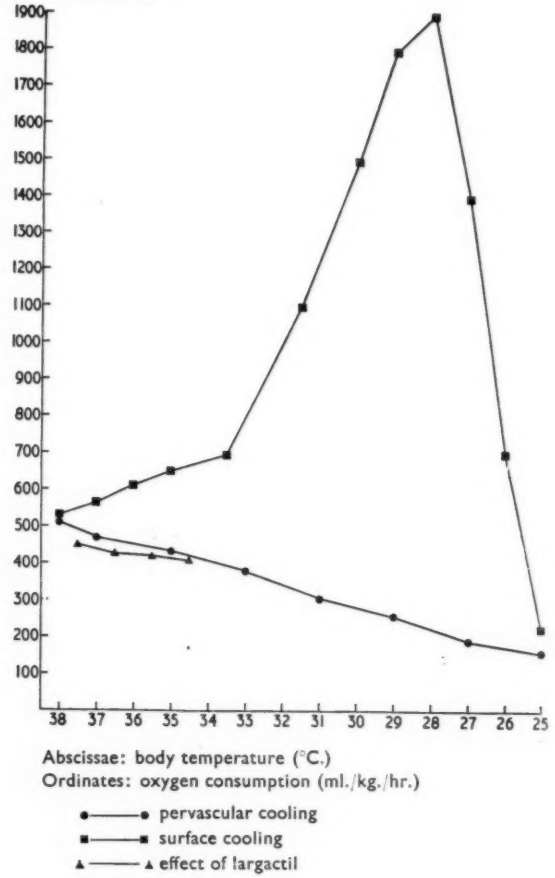
The traditional view that cold potentiates shock (Best & Taylor, 1940) is based on the clinical fact that patients in shock are made worse by exposure to cold, but, if a lowered body temperature can be obtained without inciting stress responses and requiring additional expenditure of oxygen and tissue glycogen, and other expensive bodily adjustments, resistance to shock may be increased. Experimental evidence that this is so will now be given.

3. Conclusions Based on Experiment

From experiments on over 300 dogs certain facts have been ascertained. Cooling and re-warming through the temperature range of 38°–25° C. by the pervascular method is innocuous per se. On the other hand, cardiac manipulations upon the cooled heart introduce a real hazard from ventricular fibrillation which can be reversed by electrical means in only about two-thirds of the cases. The canine heart is more liable to this complication than that of the monkey or man.

Figure 1 is a composite graph showing (i) that oxygen consumption in the anaesthetized dog cooled by the pervascular method is a linear function of temperature reduction; (ii) that oxygen consumption in an anaesthetized dog cooled by exposure to cold may show a sharp rise in the initial phase

FIG. 1. CHANGES IN OXYGEN CONSUMPTION WITH TEMPERATURE REDUCTION BROUGHT ABOUT IN DIFFERENT WAYS IN LIGHTLY ANAESTHETIZED DOGS



of cooling even while body temperature is falling; (iii) that neither body temperature nor oxygen consumption of the anaesthetized dog is significantly lowered by massive doses of largactil (chlorpromazine).

A large number of experiments were performed on dogs in which various degrees of local ischaemia were imposed at lowered body temperatures, and the effect compared with that following similar periods of ischaemia at normal body temperature. For example, bilateral renal ischaemia of four hours' duration has been shown to be uniformly lethal in the dog, uraemia developing within four to five days. Neither functional nor anatomical changes were found to follow ligation of kidney pedicles for four hours at 25° C. The clinical observation that the usual post-operative oliguria does not tend to occur when surgery has been performed under hypothermia (Rob, personal communication, 1954) suggests that the kidney under these conditions is better able to resist whatever antidiuretic effects may result from surgical trauma. Similarly, anoxic injury to the liver during hypotension, deliberate or otherwise, is less liable to result in hepatic

damage at lowered body temperatures (Delorme, unpublished observations, 1954).

Comparison of the effects of increasing amounts of air injected directly into the cerebral circulation at normal and reduced body temperatures showed increased resistance to brain damage in the presence of hypothermia (Table I). This is significant in view of the fact that cerebral air embolism is one of the present hazards of open cardiac surgery.

TABLE I. EXPERIMENTAL CEREBRAL AIR EMBOLISM BY INTRACAROTID INJECTION (DOGS)

Dosage (ml./kg. body-weight)	Effect at normal body temperature*	Effect at 25° C.
0.25	Marked neurological damage	Nil
0.5	Extensor rigidity, nystagmus	Nil
1.0	Running movements, convulsions, death after 24 hr.	Nil
2.0	Coma, death after 6–8 hr.	Temporary recovery of consciousness, no spasticity, death after 72 hr.

* From Geoghegan & Lam (1953).

This protective action may also be seen in the absence of chromatolysis in the sensitive ganglion cells of the retina after one hour of cerebral ischaemia at 25° C. (Plate VIII, fig. A). At normal body temperature this occurs within 5 min. of the onset of retinal ischaemia.

Central arrest of the circulation is a more exacting test of the value of hypothermia than any local ischaemia, and the various ways in which circulatory stasis has been produced experimentally, as well as the results of some of these procedures in the hypothermic and control animal, are given in Table II.

Observations on the oxygen content of venous blood in various parts of the circulation under normal and under hypothermic conditions are given in Table III. In every case the oxgyen content is higher in the cooled animal than in controls, particularly in the blood returning from the brain. The coronary venous blood oxygen content, however, is only slightly higher in the cooled state; this indicates that the myocardium is still able to extract oxygen in considerable amounts. In view of reduced coronary blood flow it is by no means certain that the active myocardium does in fact extract as much oxygen as it requires. Whether this is owing to an inhibition of oxidative enzyme mechanisms or to a relative deficiency in oxygen supply is not established. The fact that the myocardium seems to be the limiting factor in hypothermia in warm-blooded non-hibernating animals points to the need for a safe method of procuring a quiescent heart for open cardiac surgery under hypothermia. This has proved extraordinarily difficult to achieve in the experimental animal and only recently has some success been obtained by two different methods. One method is the selective cooling of the heart to low temperatures (7°–10° C.), by perfusing the pericardial sac with iced saline after the rest of the body has been reduced to 25° C. and inflow to the heart occluded. The second is by injecting a cholinesterase inhibitor (prostigmine) into the coronary circulation, producing a prolonged acetylcholine block which can be reversed at will by atropine. Virtually complete cessation of cardiac activity has been achieved by each of these methods for 10 min. with survival of the animal.

TABLE II. EXPERIMENTS INVOLVING GENERAL ISCHAEMIA

Nature of ischaemia	Duration (min.)	Average survival time at normal body temperature (min.)	Result at 25° C.	Number of experiments
Blood pressure: 40 mm. Hg or less	240–300	240 (15 dogs)	Full recovery	10
Occlusion of venous return to heart	20–70 (average 32)	6.5	Full recovery	54
Arterial exsanguination; blood pressure: 0–10 mm. Hg	60–150 (average 83)	5.5	Full recovery	24
Cardiotomy with intracardiac procedures	5–30 (average 13)	—	29 survivals, 12 deaths (irreversible ventricular fibrillation)	41
Chemical arrest of heart action	1–12 (average 8)	—	11 survivals, 9 deaths (irreversible ventricular fibrillation)	20
Chemical arrest plus selective cooling of heart	10–22	—	4 survivals, 1 death	5

In general, the changes that occur as a result of lowering body temperature may be summarized under two headings: those which may be expected to confer benefit upon the organism and those which are potentially dangerous.

a. Beneficial Effects of Cooling

i. *A reduced oxygen requirement* is the most obvious advantage.

ii. *The inhibition of harmful enzymic and, possibly, bacterial activity*. The former preserves the cell damaged by anoxia, while the latter prevents the proliferation of anaerobic pathogens resident in the lumen of the gut and the liver, during circulatory stasis, at least in the experimental animal. Whether

TABLE III. AVERAGE OXYGEN CONTENT OF BLOOD DURING HYPOTHERMIA

	Oxygen content at 25° C. (ml./100 ml.)	Number of dogs	Normal oxygen content at 37.5° C. (ml./100 ml.)
Arterial	20.2	13	19–21
Portal	18.8	6	15–16
Hepatic vein	17.7	5	14–15
Inferior vena cava	16.8	1	12–14
Right auricle	16.0	6	10–11
Jugular vein	19.1	2	13.8
Coronary sinus	7.3	1	6

infection from wound contamination is inhibited or not is as yet unproved, but empyema has not occurred in over 150 thoracotomies performed under clean but unsterile conditions.

iii. *A reduced cardiac activity* which reduces the work of the heart while maintaining an adequate oxygen supply to tissues.

iv. *A direct anticoagulant effect* which tends to reduce the danger from thrombosis in the presence of circulatory disturbance.

v. *A reduction in the amount of anaesthetic required.* It is possible that, eventually, deep cold narcosis in conjunction with muscle relaxant drugs may be considered adequate for surgical procedures, but as yet hypothermia as an independent anaesthetic technique has not been attempted.

b. Potential Dangers of Cooling

i. *Disturbances in acid-base equilibrium.* Unless prevented by hyperventilation, the pH of blood is lowered during cooling; this may initiate a series of changes including a rise in haematocrit readings, with a shift of water into the cell and a loss of potassium from the cell. The latter has been implicated in the increased irritability of the myocardium and the tendency to fibrillation seen in the hypoventilated, acidotic animal (Montgomery, Prevedel & Swan, 1954). Interference with oxygen dissociation by cold, or by changes in pH, does not seem to be of great significance. The simplest way of avoiding acid-base changes is to maintain an active respiratory exchange throughout the hypothermic period.

ii. *A reduction in platelets* which may be associated with a troublesome bleeding tendency occurs both in surface cooling and in any exteriorized circulation technique involving pumps or oxygenating systems. In simple arteriovenous cooling, no change in platelets occurs, but in Ross's vein-to-vein cooling a blood pump is used and some loss of platelets may occur.

iii. *Interference with hepatic and renal function* may permit dangerous accumulative effects of such substances as heparin and glucose, as well as of anaesthetic agents and the drugs commonly used in conjunction with anaesthesia.

iv. *Undesirable cardiac effects.* Certain changes in electrocardiogram pattern occur regularly in cooling, such as increase in conduction time, with prolonged PR interval and widening of the QRS complex. These appear to be harmless and ventricular fibrillation is extremely rare in the absence of cardiac manipulation, but its occurrence during experimental cardiac surgery is a serious difficulty which has not yet been entirely overcome.

v. *Undesirable responses on the part of the body to the stimulus of cooling.* These include shivering with increased oxygen requirement, and the profound endocrine activation sometimes designated as the " cold stress response ". These must be controlled by skilful anaesthetic technique or by some form of blood-stream cooling. It is interesting to note that alcohol in sufficiently large amounts may act as an adequate pre-medication to hypothermia, and many historic instances of recovery from low body temperatures due to exposure are attributable to this form of protective anaesthesia.

vi. *The danger of local cold injury*, although present, does not seem to be a serious contra-indication. Peripheral vasoneuropathy has not been described following induced hypothermia, even with surface cooling.

vii. *An " after-drop " in deep temperature*, unpredictable in degree, may occur after cessation of surface cooling when re-circulation occurs in the superficial tissues which are chilled to very low temperatures. Edholm (personal communication, 1954) has pointed out that this superficial layer to a depth of only 2 cm. makes up more than half the body mass, and that during equilibration of body heat internal body temperature may be reduced suddenly to dangerous levels.

4. Theoretical Considerations

It is clear that no full understanding of the cause of altered function during and following reductions in body temperature can be gained until the nature of changes in the individual cell can be determined. In certain tissues, such as skin, these changes are probably slight within a wide temperature range. Allen (1941) has shown that entire limbs can survive complete anoxia for more than 50 hours at 2° C., but that in other more specialized tissue a subtle derangement of function occurs; this is aggravated if the cooled tissue is kept in a relatively high state of activity as in the case of the myocardium. The effect of activity is so important that the inactive central nervous system, highly susceptible as it is to anoxia, is capable of surviving the combined effects of cold and ischaemia for much longer periods than is the working myocardium.

Extreme levels of cold (−5° to −7° C.) of short duration are non-lethal to tissues (Andjus & Smith, 1954), but a steady loss of function is noted if tissues are kept refrigerated at −5° C. for several hours. The cause of this decrement in function, i.e. in the ability of the nerve cell to conduct or the muscle cell to contract following prolonged cooling, may be bound up with the failure of the cooled cell to produce sufficient energy to maintain its osmotic relationship with extracellular electrolytes, particularly with sodium and potassium. Csapo (personal communication, 1954) has shown that the gradual loss of contractility in refrigerated uterine muscle strips may be greatly delayed by reducing the level of sodium in the Krebs solution in which they are immersed to approximately that within the cell. The short periods of moderate hypothermia now employed clinically probably do not invoke significant changes of this nature. All reports bear out the reversible nature of the functional changes in short-term cooling, but, in any attempts at " suspended animation " of intact organisms or at prolonged refrigeration of living tissue, this problem of gradual biochemical denaturation must be encountered.

That potassium disturbance in cardiac muscle is in some way concerned with the lowering of the fibrillation threshold seems established (Harris, Bisteni, Russel, Brigham & Firestone, 1954); whether it is the lowered potassium content of the cell (Swan *et al.* 1953) or locally liberated potassium acting as an exciting agent that leads to ectopic ventricular activity is uncertain. Whether the primary event is sodium entering or potassium leaving the cell is also unknown.

The paradoxical effect of high alveolar carbon dioxide in permitting survival of warm-blooded animals following reduction of body temperature far below levels which would be lethal if air or oxygen were breathed (Andjus & Smith, 1954) stresses the way in which physiological responses may be altered at lowered body temperature. Hypercapnia during hypothermia seems to act as a peculiarly innocuous metabolic depressant.

It is now widely accepted that the body reacts with a non-specific stress response to many different forms of stimulus or injury. One of these is exposure to cold. The pituitary-adrenal system is involved and, within a few hours of exposure to severe cold, depletion of adrenocortical lipids and ascorbic

acid may be detected (Sayers & Sayers, 1949 ; Hemphill & Reiss, 1947). A fall in eosinophils is regularly seen in surface cooling and an initial rise in blood sugar suggests adrenaline release. These changes are not seen when animals are cooled through the blood stream, possibly owing to absence of surface stimulation and to direct and early depression of endocrine and neural mechanisms by cold.

Claude Bernard saw disease as the outcome of attempts to maintain the constancy of the internal environment in the face of noxious influences where the adaptive responses, although appropriate in kind, are excessive in degree. Swan (1954) in an editorial on hypothermia speaks of the need for " acquiescence to environment at a diminished metabolic level " in order to reduce the cost of maintaining homeostasis. This may emerge as a new concept in the therapy of trauma or disease whereby chances for ultimate recovery are enhanced by restraining the immediate and perhaps inordinate efforts of the body to adjust itself to hurtful impositions from the external environment.

Proof of the clinical value of hypothermic techniques does not depend upon comparative clinical trials as with most new forms of therapy but may be considered to be established by the salvage of even a small number of lives otherwise forfeit. At the same time it remains experimental in the sense that its use should be limited to exceptional clinical circumstances and to places where there is available a staff trained in its technique, aware of its dangers and equipped for further investigation into its many unexplored aspects.

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PRACTICAL APPLICATIONS OF HYPOTHERMIA

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1 Indications
2 Underlying principles
3 Apparatus
4 Technique of cooling
5 Future developments
References

As a result of the world-wide interest in hypothermia and the considerable amount of experimental work which it has stimulated, it is now possible to assess some of its applications and possible hazards in relation to clinical cases.

For the purposes of this communication, hypothermia means the reduction of body temperature to the region of 25° C. in order to reduce cellular metabolism and oxygen requirements. As far as we have been concerned in the Thoracic Unit at Guy's Hospital, this has been with the sole object of providing a bloodless surgical field in order to perform some manoeuvre which is technically impossible or inadequately performed with existing closed techniques. We have not been concerned with minor reductions of temperature as an aid to anaesthesia.

1. Indications

Our indications for hypothermia at present are therefore limited. The most suitable application is, we feel, in the closure of septal defects, particularly atrial septal defects. Ventricular septal defects present more formidable problems, but the closure of the ventricular septal defect—as part of the treatment of Fallot's tetralogy—is certainly technically possible now.

The resection of an infundibular stenosis under direct vision is a short procedure and the obstructing supraventricular crest can be easily and accurately excised with scissors. This seems more satisfactory than blind infundibular resection, and it is likely to supersede the latter method which has paved the way to an open approach to the stenosis.

The results of closed mitral valve surgery are so satisfactory that there does not seem to be an indication for present hypothermic techniques in this field. On the other hand, the surgery of the tricuspid valve is more difficult and it may well benefit from the advantages of surgery under direct vision.

There have been reports of the reconstruction of stenosed pulmonary valves through the open pulmonary artery (Swan & Zeavin, 1954), which enables refashioning of the valve cusps under vision. Some of these operations have been followed by pulmonary incompetence, and we have been content to continue with the closed method of valvotomy for the present. Hypothermia may make it possible to do a more accurate reconstruction of the cusps in aortic stenosis or it may be possible to replace the valve with some prosthesis if coronary air embolism can be avoided; but this would appear to be far in the future.

Surgical abnormalities of the great vessels, including aorto-pulmonary defect, aneurysms of the arch and descending aorta, complicated coarctations and recanalized ducti, can now be approached with more confidence during hypothermia. This results from the ability to clamp across major vessels in order to deal with haemorrhage or to prevent it.

2. Underlying Principles

In order to provide a sound experimental background to the work and to develop apparatus and techniques, about 200 dogs have been subjected to hypothermia. At the same time physiological observations have been made which are helping to piece together the altered physiology of the cold non-hibernator (Ross, 1954a; Swan, Zeavin & Blount, 1953; Swan, Zeavin, Holmes & Montgomery, 1953). It is in this field that further work must be directed to establish the limits of safety of the technique. As a result of these experimental and physiological investigations, we have been able to establish a number of principles which are of practical application in human subjects.

i. The period of cooling and maintained hypothermia should be as short as possible in order to prevent the reversible physiological changes of hypothermia being superseded by irreversible pathology.

ii. The causes of ventricular fibrillation under conditions of hypothermia are likely to be similar to those which operate at normal temperatures, namely, a poor coronary circulation associated with various exciting stimuli—either traumatic, nervous or biochemical. Therefore every effort should be directed towards ensuring an adequate well-oxygenated coronary blood flow; this, in effect, amounts to maintaining an effective blood pressure. At the same time, unnecessary stimulation of the cold heart should be avoided, and this includes the local heating of the heart by powerful operating-theatre lights. Additional factors increasing myocardial irritability, such as anoxia and hypercapnia, can be minimized by the anaesthetist's paying careful attention to the maintenance of adequate ventilation.

iii. The heart should be exposed and available for observation before cooling is begun. This means that signs of irregularity or irritability are immediately detected and, if necessary, massage or defibrillation can be applied without delay.

iv. It appears safe to exclude the heart and brain from the circulation for about 10 min. at 25° C. and for correspondingly shorter periods at higher temperatures.

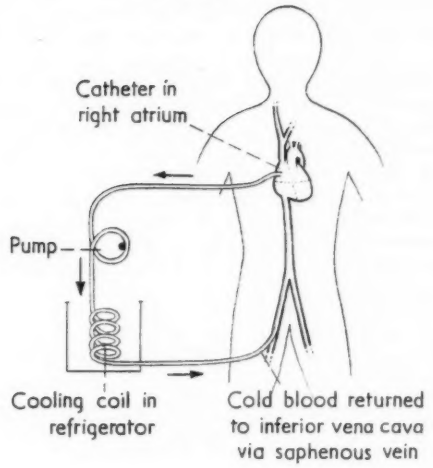
v. A completely dry heart is possible only if all the veins entering both the left and right heart chambers are clamped, and one has to keep in mind the possibility of a left or double vena cava in a congenital heart lesion.

vi. Coronary air embolism must be guarded against by keeping the septal defect uppermost and by flooding the heart chambers or the whole chest before closing the heart wound.

vii. After the cardiotomy, the veins should be released slowly in order to allow the heart to reaccommodate itself to the burden of pumping.

viii. Re-warming should not be delayed but should begin immediately the period of exclusion of the heart from the circulation is over. Warm saline applied locally to the myocardium is often useful at this stage to improve the beat.

FIG. 1. DIAGRAMMATIC REPRESENTATION OF VENOUS COOLING


Catheter in right atrium
Pump
Cooling coil in refrigerator
Cold blood returned to inferior vena cava via saphenous vein

3. Apparatus

An early decision in this work was the use of blood-stream cooling, since it permits accurate control of the rate and depth of cooling. In addition it allows rapid cooling while the chest is open. The method of blood-stream cooling we have evolved (Ross, 1954b; Brock & Ross, 1955) cools only the venous blood, and in this way avoids damage to major arteries and the introduction of an artificial arteriovenous fistula (fig. 1).

The apparatus consists of a plastic blood-containing coil surrounded by a refrigerant solution. For the sake of convenience and sterility, it has been elaborated and now consists of a separate refrigerator unit outside the operating theatre and a blood-cooling coil and pump in a compact unit next to the patient. (See figs. 2, 3.)

FIG. 2. HAND-DRIVEN ROTARY PUMP AND REFRIGERANT TANK CONTAINING PLASTIC COOLING COIL

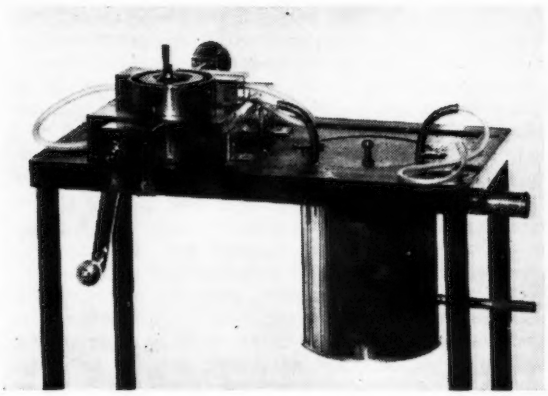
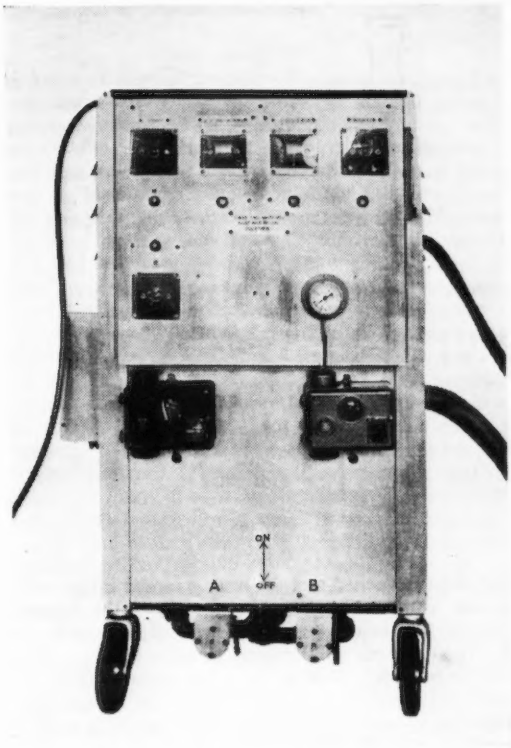


FIG. 3. REFRIGERATOR UNIT WHICH SUPPLIES A SOURCE OF REFRIGERANT AT −4° C.



4. Technique of Cooling

With regard to the selection of patients, we have not noticed any particular hazards associated with the cooling of patients in the older age-group nor an increased tendency to ventricular fibrillation in older patients.

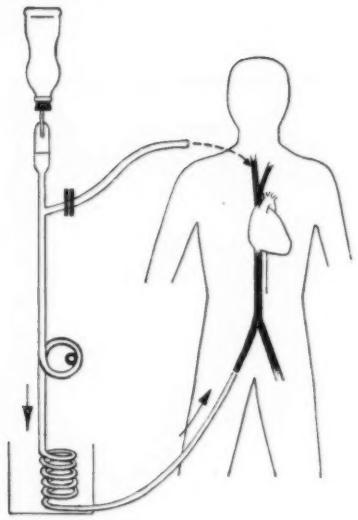
The pre-operative blood picture and electrolyte levels are established in order to compare with the pictures during cooling and after operation, and a search is made for cold agglutinins.

The pre-medication and anaesthesia are conventional, but the anaesthetist is encouraged to ventilate fully except during the period of circulatory arrest.

The most convenient position of the patient is on his back, and a double submammary incision with transverse sternotomy gives good access to the great vessels on both sides of the heart. Continuous electrocardiographic tracings are taken throughout the procedure. Temperatures are recorded by means of an ordinary glass thermometer inserted into the pharynx; in addition, a sterile thermometer is available with which the surgeon can take direct readings of the temperature within the chest.

Immediately the patient is anaesthetized, a small incision is made over the saphenous vein in the femoral triangle, which is cannulated and connected to the return end of the blood-cooling coil. The other limb of the coil is connected to a bottle of saline, and the system acts as a conventional intravenous drip during the thoracotomy. (See fig. 4.)

FIG. 4. DIAGRAM OF COOLING CIRCUIT AS IT IS INITIALLY SET UP TO ACT AS AN INTRAVENOUS DRIP (cf. fig. 1)



The chest is opened and the heart exposed in the ordinary way and at normal temperature, without the hazards of ventricular fibrillation or of hypothermic alterations in physiology. The diagnosis of the heart lesion can be rapidly confirmed by palpation or by manometric pressure tracings, and a decision to cool can be made at this stage. If hypothermia is required, a small hole is made in the tip of the right atrial appendage, and the afferent limb of the cooling coil is disconnected from the saline drip and pushed up through this incision into the superior vena cava (Plate VIII, fig. B, facing p. 221). The pump is then set in motion, and cool venous blood is returned to the saphenous vein while the operation proceeds. The surgeon prepares the great vessels for their subsequent clamp-off, and by the time rubber snares have been threaded around the vessels there will generally have been a considerable fall of temperature. If it is not low enough, it should not be necessary to wait more than about 15 min. before proceeding with the cardiotomy. The total cooling-time is likely to be about 45 min., depending on the size of the patient. The blood pressure is maintained above 80 mm. Hg during this phase with the aid of a noradrenaline drip.

As soon as the temperature has fallen to the desired level, the veins are occluded, and a soft clamp is placed across the pulmonary artery and aorta. The anaesthetist stops ventilating and keeps a check on the time of circulatory arrest. An incision is made in the atrium or ventricle, and any blood obscuring the defect is cleared by suction. After the defect is dealt with, the exposed chamber is kept flooded with saline while the incision is closed. The veins are then released in turn and re-warming is started. The patient is allowed to re-warm to about 35° C. or until consciousness returns, before being returned to the ward.

If ventricular fibrillation occurs during the period of cooling, the cooling is temporarily stopped and the already exposed heart is massaged and electrically defibrillated if necessary. If fibrillation occurs when the temperature is low, or during the period of clamp-off, the intracardiac procedure is completed as planned, and the circulation is restored before any attempt is made to defibrillate. If myocardial tone cannot be restored by massage alone, warm saline on the myocardium, together with intracardiac adrenaline or calcium chloride, may be effective (Milstein & Brock, 1954).

5. Future Developments

The wider application of hypothermia to problems of cardiac surgery will depend upon the provision of a longer period of surgery within the dry heart. This may be achieved by the assistance of an artificial circulation or by cooling to lower levels. With a differential cooling technique, we have been able to cool dogs' hearts and brains down to about 12° C., with asystole for 30 min., but the method has not yet been applied to man.

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CARDIAC RESUSCITATION

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1 Preliminary considerations
  a Aetiology
  b Prophylaxis
  c Diagnosis and treatment
2 Cardiac standstill or asystole
3 Ventricular fibrillation
  a Electrical defibrillation
  b Defibrillation by potassium
  c Defibrillation by massage and drugs
  d Spontaneous defibrillation
4 Technical
  a Duration of shock
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  d Drug administration
5 Artificial pace-makers
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Cardiac resuscitation is the starting of an effective heart beat in a heart which has stopped, usually in the course of an operation (i.e. cardiac arrest) and this may occur in two ways: firstly, cardiac standstill or asystole, where no movement of any kind can be seen or felt; secondly, ventricular fibrillation, where the whole heart is undergoing small unco-ordinated muscular contractions involving only a small group of fibres in any one place at a particular time, while a neighbouring area may be in the refractory period. The classical description is that the heart looks and feels like a " bag of worms ". No blood is ejected from the heart in either state.

It is the purpose of this article to discuss the treatment of these two conditions.

1. Preliminary Considerations

Sudden cardiac arrest on the operating-table is as old as surgery itself, and in particular it has been observed and reported since the introduction of anaesthesia in the nineteenth century. However, the problem has been more noticeable with the advent of modern cardiac surgery, and in recent years a great deal of work has been done on this subject, particularly in the United States of America. But it must be emphasized that cardiac arrest can occur at any time in any operation and that, as its treatment is relatively easy, a simple plan should have been worked out beforehand for dealing with this emergency, as speed in starting treatment is vital and special training in thoracic surgery is not needed to carry it out.

Unlike other procedures, fortunately it takes a long time to acquire statistics; but although the percentage survival is low, it is a worth-while result in what until recently has been considered a terminal event. Johnson & Kirby (1951, 1954) from the Hospital of the University of Pennsylvania cite 10 survivors out of 19 (7 out of 13 in cases of non-cardiac disease) over a 7-year period, with an incidence of cardiac arrest of one in 7,500 in all types of anaesthesia and one in 5,000 in general and spinal anaesthesia.

a. Aetiology

The cause of the vast majority of cases of cardiac arrest is hypoxia, and no form of treatment is of any use unless the hypoxia is remedied. This is commonly caused by varying degrees of obstruction to the airway or by inadequate pulmonary ventilation, and is particularly important in patients undergoing lung operations, or in those with any form of cardiac lesion. Obviously, an overdose of an anaesthetic agent, leading to respiratory depression or paralysis, will have the same effect.

Direct operations on the heart, particularly those requiring a ventricular incision, will produce arrhythmias which can and do lead to ventricular fibrillation. In these cases the heart is abnormal, with congenital defects or with valvular and myocardial lesions, and is therefore all the more susceptible to hypoxia. Coronary thromboses and air emboli are obvious causes and they carry a very poor prognosis. The next important group is that caused by severe haemorrhage leading to prolonged hypotension; usually in these cases the heart stops beating and is found in asystole when the chest is opened.

Lastly, there is a group of cases in which cardiac arrest follows procedures such as incising the pleura, and in which it is considered a reflex, usually of vagal origin. These cases are rare but should be treated in exactly the same way.

There are obviously other precipitating causes but most commonly one or other of the above causes is found, particularly in association with hypoxia.

b. Prophylaxis

A good anaesthetic, with adequate oxygenation all the time, is the best preventive measure. In cardiac surgery the appearance of the heart itself, as regards its colour (i.e. blue from hypoxia) and the type of beat, are useful warning signs. In addition, if an electrocardiogram is being taken, any changes in rhythm—and particularly ventricular extrasystoles—are useful warning signs. The use of intravenous procaine or pronestyl has not produced any marked benefit, but again it is difficult to obtain controlled observations. The injection of local anaesthetics into the myocardium in the area where the incision is made appears to be the most helpful use of these drugs. Instillation of local anaesthetic into the pericardial cavity before it is opened is used by many surgeons to reduce irritability of the heart.

It is vitally important to maintain an adequate blood pressure throughout the operation.

c. Diagnosis and Treatment

Early and correct diagnosis is by far the most important factor in relation to survival. This means that the condition should be recognized and treatment started within two minutes, with a maximum of four minutes to avoid brain damage. The heart will stop in one of two ways—asystole or ventricular fibrillation, and the two must be differentiated as their treatment is different. The only sure way to diagnose either condition is by direct palpation or inspection of the heart.

An electrocardiogram, if available, is helpful and will always demonstrate ventricular fibrillation if this is present, but a heart that has stopped putting out an effective stroke volume may still produce regular complexes, and this type of heart should also be resuscitated.

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The moment the anaesthetist reports that no pulse or blood-pressure reading is obtainable, the surgeon should start to confirm this by palpating any major vessel available and by rapid auscultation. If the chest is already open, the state of the heart can be observed at once. If not, the patient should have the chest exposed and an incision made in the left fourth or fifth interspace from the left sternal border to the mid-axillary line as soon as the anaesthetist has started positive pressure breathing with 100% oxygen. This requires only one instrument, a scalpel, and calls for no special experience except sufficient to avoid incising the lung when the pleura is opened.

If a rib spreader is available it is inserted, the lungs retracted and the heart examined. If this instrument is not available, exposure can be obtained by cutting one or two of the costal cartilages. It is advisable to open the pericardium if there is ventricular fibrillation, as it is then easier to massage the heart directly and also to apply the electrodes to its surface. If the heart is in asystole, massage with an intact pericardium can be tried first, but if this is not successful the pericardium should be opened without hesitation. This incision can be made in a minute at the most and bleeding is obviously no problem. No time should be spent on skin preparation or in fixing towels, and the initiation of treatment should outweigh every other consideration.

The only exception to this rule is if an abdominal incision is already present. In this case the aorta can easily be palpated, or the heart itself through the diaphragm, to confirm the diagnosis. The heart can be massaged by compressing it between the diaphragm and chest wall or by incising the diaphragm, entering the pericardial cavity, and massaging the heart direct (Bailey, 1941). Transdiaphragmatic massage is not as efficient as transthoracic owing to the position, and it is more tiring to perform. If an effective beat is not restored promptly, it is better to open the chest as previously described.

The anaesthetist's function is to maintain artificial respiration with pure oxygen. If an intratracheal tube is being used, this is easy; but if not, a close-fitting face mask will do for the immediate emergency period and this can be changed to a tube when the heart has been re-started, or earlier, as long as it is put in quickly. If no other method is available, mouth-to-mouth breathing can be used to inflate the lungs for a short while.

Other methods of diagnosis, such as prolonged auscultation and retinoscopy, are useless and time consuming. Whether the heart is in asystole or fibrillation, the first step is to start rhythmic cardiac massage and, once this is started, preparation for further treatment can be made.

2. Cardiac Standstill or Asystole

This is the commoner of the two ways that the heart stops at operation. The guiding principle in treatment of this condition is to maintain a flow of blood to the coronary vessels and the brain, which is sufficient to maintain oxygenation of these tissues and so avoid irreparable damage. The only effective way to do this is by cardiac massage, and it should be possible in this way to produce an aortic systolic pressure between 50 mm. and 70 mm. Hg (Deuchar & Venner, 1953). This was first done by Schiff in 1874, as quoted by Cutler (1947).

The advantage of a chest incision is that it is the only one in which massage can be carried out effectively for more than a short period. To do this it is preferable to use both hands, alternately compressing and releasing the ventricles between them. The aim is to empty the ventricles by compression and then allow time for relaxation and filling. Care must be taken not to compress with the finger tips but rather with the flat of the hand, as it is quite easy to perforate an atrium with a finger tip. Efficient one-handed massage can be continued for only a short time, and, with the fingers on one side and thumb on the other, emptying of the ventricles is hard to obtain. It is best to open the pericardium and massage the heart direct in order to obtain the maximal aortic pressure possible (Beck & Mautz, 1937). There should always be a relief for the surgeon as he will need a rest after about five minutes, and an alternation every five minutes for as long as is necessary allows two people to keep up effective massage for long periods.

When massage is started, several things may happen. Firstly, the heart may start up on its own after a short period, particularly if the time of arrest has been short. If the heart does this and starts to maintain an adequate blood pressure, no more need be done except to watch it for a while before sewing up, to make sure no return of standstill occurs.

Sometimes the heart starts again but the blood pressure remains too low (40 mm. Hg), and we have found (McMillan, Cockett & Styles, 1952) that, if it is raised artificially by means of a vasopressor such as noradrenaline or a longer-acting compound, this may well tide the heart over a critical period lasting several hours.

While the massage is being carried out it is an advantage, if possible, to clamp the aorta beyond the left subclavian artery so that the available blood ejected is directed to the coronary arteries and head. If the aorta cannot be reached, noradrenaline (4 ml. of 20 µg./ml.) given intracardially may help and is less likely to cause fibrillation than adrenaline.

The second possibility is that the heart will not re-start in spite of massage, and here the question of drugs to stimulate the myocardium must be considered.

The first and most widely used is adrenaline given intracardially. Kay (1951) recommends 0.1–0.2 ml. of 1:1,000 adrenaline, and Beecher & Linton (1947) say not more than 0.3 ml. of 1:1,000, as larger doses lead to fibrillation. It is best to avoid the ventricles if possible and inject it into the left auricle or into the ascending aorta proximal to a clamp, so that the drug is pumped by massage into the coronary vessels and can act rapidly.

The second drug is calcium given in the form of calcium chloride (2 ml. of a 10% solution), repeated if necessary; this has a dramatic effect in restoring tone to the flabby arrested heart and will often re-start it at once or after some more massage (Kay & Blalock, 1951). The disadvantage of both these drugs, and particularly of adrenaline, is that it may well convert ventricular standstill to ventricular fibrillation by starting up multiple pace-makers (Wiggers, 1940); the treatment of ventricular fibrillation is described subsequently. In view of this risk, again increased by anoxia if present, these drugs should not be used until massage alone has failed.

Rapid transfusion may help as long as the potassium content of the transfused blood is not too high. Where haemorrhage is the cause of cardiac arrest, rapid blood transfusion is a necessity to restore blood volume. This is best given intra-arterially (intra-aortically, if possible) if the heart is stopped, or intravenously when the beat is re-established.

To sum up, the treatment of asystole or standstill is vigorous cardiac massage until a regular effective beat is restored. This

should be continuous even while other measures—such as the injection of drugs—are being carried out. A compression rate of between 50 and 90 is best, and ideally it is as fast as possible compatible with adequate filling.

3. Ventricular Fibrillation

There are three possible methods of dealing with this condition.

a. Electrical Defibrillation

This, to date, is the most effective and widely used method, and its possibilities were first reported by Prevost & Battelli in 1899. But it is to the pioneer work of Hooker, Kouwenhoven & Langworthy (1933) and Wiggers (1936) that we owe the success that has been achieved by this method.

The principle of electrical defibrillation is to pass through the heart a shock powerful enough to make all its muscle fibres refractory at one time, and then by massage to re-start a co-ordinated beat. Again it must be stressed that the primary treatment of ventricular fibrillation is cardiac massage to produce adequate oxygenation, and until this is done no form of treatment will be effective. This is borne out by the ease with which it is possible to defibrillate the heart of a patient connected to an artificial heart-lung machine which is supplying oxygenated blood to the coronary arteries (McMillan, Melrose & Cleland, unpublished observations).

There is a great deal of discussion about the technique of defibrillation, but the basic principle is as described above, and cases have been reported of successful electrical defibrillation, drawing the current direct from the mains supply with the most primitive of apparatus. It is known experimentally that low-voltage currents will cause ventricular fibrillation, and 110 v is the generally accepted minimum in practice for successful defibrillation; 130 v is usually sufficient for a normal heart that has stopped—and especially for a child's heart—but voltages up to at least 250 (McMillan *et al.* 1952) may on occasion be required, particularly if the heart is enlarged (owing to its high electrical resistance) and has been stopped for some time.

The technique is as follows: when the heart has been massaged for several minutes its colour will have improved (from blue to red) and usually the surgeon will notice an increase of tone in the organ as a whole. The quality of the fibrillation may also change from the slow undulation of the anoxic fibrillating heart to more rapid small fibrillary twitchings, but this is inconstant, and the best guide is the colour and tone.

When this point has been reached, the electrodes are applied on opposite sides of the ventricles so that the current passes between these through the heart. Usually one shock is given, first at about 130 v (50 cyc./sec.). This will usually stop the heart. It will then either start beating at once or following some more massage, or beat once or twice and then revert to fibrillation. If it continues to fibrillate, it should be massaged again for a minute or two and the process repeated. If, after the second shock, fibrillation is still present, then the serial defibrillation method of Wiggers (1940) should be tried. This consists of giving a series of shocks at about 0.5–1 sec. intervals, usually between five and seven shocks, and it is believed that in this way there is more chance of making all the muscle fibres refractory at once. If there is still no success from this treatment, the voltage should be increased in steps of about 30 v and the process repeated even with voltages as high as 220–250, as said before. An adequate period of massage must intervene between each attempt at defibrillation.

b. Defibrillation by Potassium

This was first demonstrated by d'Halluin (1904) and repeated by Hooker in 1929. It is undoubtedly effective, but often the dose of potassium (5 ml. of 4% potassium chloride) required will, when arrest has been produced, make it very difficult to start the heart beat again, even with the use of calcium chloride or adrenaline. Its use has been suggested recently by Swan, Zeavin, Holmes & Montgomery (1953) in hypothermia[1], and in their hands it is more effective at low temperatures than electrical methods; but here again the same general principles apply in resuscitating the heart as apply at normal temperatures. Potassium should always be available for trial, if electrical methods fail or are unavailable. Several doses may be required to be given intra-aortically or intracardially, and it may take up to a minute for the fibrillation to stop after potassium is given.

c. Defibrillation by Massage and Drugs

It is possible to defibrillate hearts by adequate massage combined with the use of intracardiac procaine (10 ml. of a 1% solution) injected into the auricle (Leeds, 1953). This process may take as long as an hour, and has the disadvantage that if too much procaine is used to arrest the fibrillation it may again be difficult to re-start the heart (Wégria, Frank, Wang, Misrahy, Miller & Kornfeld, 1953). Secondly, massage inflicts a recognizable amount of trauma on the heart, in the form of subepicardial and subendocardial haemorrhages, which it is desirable to avoid if possible. Procaine amide hydrochloride (100–200 mg.) injected into the auricle is also used.

The above three methods have disadvantages, but the surgeon should be prepared to try all of them; and the important factor is to have a regular routine that can be put into operation at a moment's notice.

d. Spontaneous Defibrillation

This is rare, but in valvular disease of the heart, particularly aortic stenosis, fibrillation may arise when an attempt is being made to split the valve, through a temporary further impairment of an already poor coronary blood flow caused by the instrument blocking the valve orifice. In these cases most surgeons complete the valvotomy; and then massage alone may produce a regular beat again after the coronary supply has been improved. If not, the usual system of electrical defibrillation is used.

Milstein & Brock (1954) have described their experiences with 30 patients who developed ventricular fibrillation during cardiac operations. Nine of these patients recovered.

4. Technical

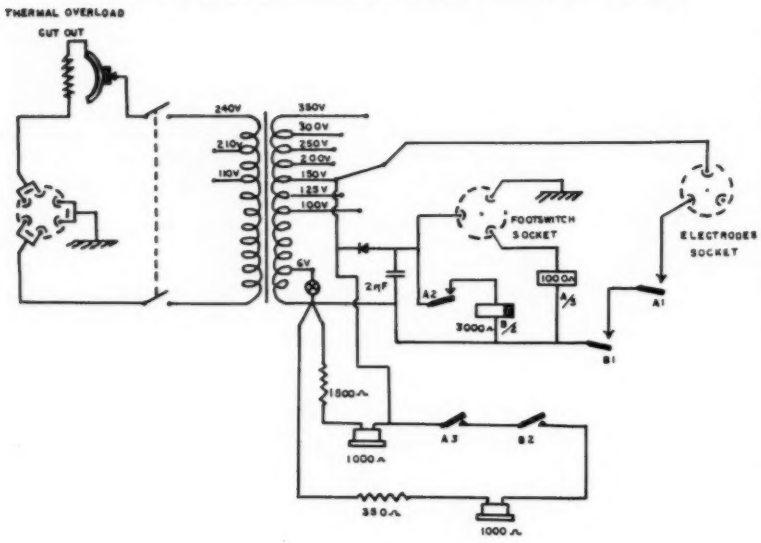
The number of defibrillators designed and in use is very great: fig. 1 shows the circuit diagram of the model at present used in St Thomas's Hospital, London. It can give shocks from 120 v to 300 v in steps, with a duration of 0.2 sec. The foot-switch will give only one shock for each separate depression and so eliminates the risk of a long duration shock. Serial shocks are given as a series of separate switch depressions.

a. Duration of Shock

In different articles, the duration is said to be anything from 0.1 sec. to 1 sec. In our experience, having used the range from

[1] See papers by Delorme (p. 221) and Ross (p. 226) of this number of the Bulletin.—Ed.

FIG. 1. CIRCUIT DIAGRAM OF CARDIAC DEFIBRILLATOR



0.1 sec. to 0.5 sec., we have finally settled arbitrarily on 0.2 sec.; 0.1 sec. appears to be too short to be effective unless high voltages are used; 0.5 sec. is long enough for electrical burns to be produced.

b. Type of Electrode and Current

The bigger the electrode the better, and we use a pair with a concave inner surface to fit the curve of the ventricle, about 2 in. in diameter[2]. If the rim of this has a series of small holes drilled around it, it is easy to sew on the inside about four thicknesses of surgical gauze, which is easily changed. (This is the method in our present defibrillator.) If the electrode and gauze are soaked in saline before use, a much better contact with the heart is obtained, a lower voltage is required and the chances of burning the heart are further reduced (Leeds, 1953). At all costs, small areas of contact such as the rim of the electrode should be avoided as this is a fruitful source of burns. We have found a scissor-type handle a help in maintaining good contact. However, Kay & Blalock (1951) prefer small electrodes arranged to give a shock in the long axis of the heart.

If the voltage settings previously described are used, a current of from 1 A to 3.5 A is passed through the heart.

[2] 1 in. = 2.54 cm.—ED.

c. Safety of Operator

All machines should have a foot-switch so that the surgeon applying the electrodes is the only person who can give a shock; the handles of the electrodes should be well insulated; and a double-wound isolating transformer to avoid the possibility of getting a shock straight from the mains is desirable. It is best for no one else to be touching the table or patient. The surgeon, the anaesthetist and at least one other member of the operating staff should be familiar with the technique, be able to use the defibrillator, and to use the correct drugs.

d. Drug Administration

Drugs used in treating cardiac arrest must be injected into the aorta or the heart itself. The intravenous route is quite useless till a heart beat is re-established. The ascending aorta proximal to a clamp about 1–1½ in. distal to the aortic valve is the best (the clamp is only in position for the injection), followed by the left auricle, pulmonary artery, right auricle and lastly, owing to their irritability, the ventricles. If the ventricles are the only easily accessible sites, they must be used rather than time being wasted in exposing other sites.

Numerous other drugs have been suggested for the treatment of cardiac standstill and ventricular fibrillation, and the field is too big for the literature to be covered adequately in this article. Nor is the use of drugs to deal with arrhythmias arising before operation or during operation, other than cardiac arrest, considered, as this is properly the field of the cardiologist and anaesthetist.

In cardiac operations, if an arrhythmia occurs during surgical procedures upon the heart, it will usually revert at the end of this period; this will also occur if any hypoxia present is remedied. If it is possible to stop the surgical procedure for a few minutes, the arrhythmia will often disappear and then the operation can be continued.

5. Artificial Pace-Makers

Bigelow & Callaghan (1951) amongst others have reported on the use of artificial pace-makers in cardiac standstill, but this work is only experimental so far although it appears promising. Zoll (1952) has reported on the successful resuscitation of patients with cardiac arrest in Stokes-Adams attacks by external electrodes, without opening the chest.

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CROSS-CIRCULATION

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1 Definitions
2 Development of the donor-circulation principle
  a Venous return: limiting factor
3 Donor-circulation experiments
  a Experimental intracardiac surgery
  b Donor circulation in human intracardiac surgery
References

The purpose of this paper is a brief description of the "donor-circulation principle" in its experimental and human applications to intracardiac surgery. The relationship of donor circulation to cross-circulation will become clearer as the definitions below are studied.

1. Definitions

The term "cross-circulation" indicates a heterogeneous group of experimental and therapeutic procedures, loosely linked by one common factor: that blood is exchanged between two bodies, or parts thereof. The means of attaining this aim, and the purposes served by the exchange, differ widely. Used in this way to embrace so diverse a group of procedures, the term becomes almost meaningless. Unfortunately it appears in the literature applied indiscriminately to each of these very distinct techniques. Such a lack of particularization can only lead to confusion.

At first sight it might appear that a donor circulation is only a particular instance of either direct or indirect, artery-to-artery, vein-to-vein, continuous exchange. The object to be served in each case is quite distinct and vastly different. The donor circulation is to supply "the support of a whole circulation" to the second animal. This latter is evidently one in which the present circulatory apparatus is not capable of maintaining life, or full function. In the particular case with which this work is concerned the reason for requiring such support is that the heart and lungs of the second animal are to be excluded, to permit operation within the chambers of the heart.

The words, "whole circulation", are significant, and convey the important fact that not only is an effective quantity of blood to be supplied, at pressure, but that blood is to be fully oxygenated, and biochemically and biophysically efficient as well as bacteriologically pure. Moreover, the blood returning via the venous connexion to the donor's body, and exhausted by passage through the recipient's body, is to have its biological balance readjusted as it passes through the vital organs of the donor's body. The donor circulation is clearly a very special application of the cross-circulation principle.

2. Development of the Donor-Circulation Principle

Indirect donor circulation is probably the only modification of the cross-circulation principle that is applicable to intracardiac surgery. This paper is concerned only with a report of the development and present position of that application.

Except where otherwise mentioned, the facts stated come from the work of Andreasen & Watson (1952, 1953a, 1953b), carried out at the Buckston Browne Surgical Research Farm of the Royal College of Surgeons of England, between 1949 and 1953. Until the recently published reports of Cohen and Lillehei (Cohen & Lillehei, 1954a, 1954b; Cohen, Warden & Lillehei, 1954), there was no other published work in the field.

In 1939, O'Shaughnessy had begun experiments with the object of operating eventually inside the chambers of the heart. He showed that, whilst clamping the cavae was fatal in about 8 min., if the clamps were removed within 4 min. a number of animals survived. William Harvey had shown how clamping the entering vessels to a snake's heart caused the heart to empty and also that if the obstruction was removed in time, the heart refilled, and life continued in the animal. Together with the fact that it is some minutes before life is extinct, even with total obstruction to the venous return, it is well known that a patient can manage quite well, for a time, after a serious haemorrhage, living on a very small residual blood volume. In 1947, Opdyke & Foreman, studying the effects of quantitative losses of blood by haemorrhage, showed that a reduction of coronary flows to 30–60% of that in controls was quite compatible with life and recovery of the animals. In the same year, Eckstein, Graham, Liebow & Wiggers published a study of the changes occurring after measured reductions of the blood flowing back to the heart from the inferior vena cava after haemorrhage, showing that life could be maintained and recovery expected with a minimal critical flow of 150 ml./min. The idea suggested was that it might be possible to reduce artificially, in some way, the flow of blood through a body so as to permit opening the heart, after suitable by-passing of that organ.

The fact that the opening of the heart would necessitate exclusion of the lungs as well, pointed directly to the need of a second circulation on which the rest of the animal's body, as well as the nourishment of the myocardium and lung tissue, would be maintained. The most natural second circulation which could be obtained would be that of another animal. Use of a second animal to supply a continuous flow of blood is a not uncommon physiological experiment, and this has recently been applied in man for therapeutic purposes (Bierman, Bryon, Kelly, Dod & Black, 1951; Salisbury, 1949; Duncan, Tocantins & Cottle, 1940). All things considered, there seemed no reason why an attempt should not be made to extend the principle to the particular instance where an animal, because of exclusion of its own heart and lungs, requires the support of a whole circulation with all its attributes.

On setting this idea alongside the many ingenious attempts which had been made to answer the problem by means of mechanical pump oxygenators, it seemed that the comparison was much in favour of the cross-circulation experiment. Given control of such a system, the problems of oxygenation, removal of metabolites, regulation of pH, balance of liver and kidney functions, removal of particulate matter by the lung filter, etc., would possibly be nearer solution, since it might reasonably be expected that the supporting animal would supply these functions on behalf of the recipient. Again, the fact that support would be required for a period of only 30 min., or 40 min. at the most, during the

actual exclusion of the recipient's heart and lungs, seemed to add to the hope that the donor's system would stand the extra strain put upon it.

Since the size of flow demanded from a donor must be within compass of its own circulation, without detriment to itself, a study of the minimal flows that would maintain life was undertaken. The average accepted minimum cardiac output of an anaesthetized dog was said to be about 165 ml./kg./min., but the work of Eckstein *et al.* (1947) seemed to suggest that it might be much lower, i.e. 12–15 ml./kg./min., at least for short periods. Working on blood oxygenator pumps, Stokes & Gibbon (1950) stated that the minimum satisfactory flow for maintenance of life was 99 ml./kg./min. By clamping the superior cava cephalad to the entrance of the azygos vein, and the inferior cava close to the heart, Andreasen & Watson (1952) maintained life, with recovery in a significantly high percentage of animals, on a measured flow of 80–90 ml./min., i.e. 8–9 ml./kg./min., in their series. Cohen and his co-workers (Cohen & Lillehei, 1954a, 1954b; Cohen *et al.* 1954) in a most careful study, using a direct measurement of flow, were able to confirm the findings of Andreasen & Watson (1952) and to fix the critical survival flow, or "azygos factor", at 8–14 ml./kg./min.

On flows of the azygos factor range, the blood pressure sinks to 30–40 mm. Hg; respiration is slow but regular; the heart contracts down, beats at about 40/min., but contractions are smart; pupils dilate and are sluggish. After 30 min., re-establishment of the circulation is quite possible but requires care, as, if the cavae are released too quickly, the heart overfills and stops, or fibrillates; 80–90 ml./min. was not deemed satisfactory from the recipient's point of view.

By drawing the venous return direct from the femoral vein, through a small pump, into the cannulated cardiac end of the azygos vein, flows of different volume could be supplied merely by regulating the speed of the pump. When the flow had been increased to 150 ml./min., the animal seemed, as it were, to come to life. The blood pressure rose sharply to 100 mm. Hg, respiration became normal, as did heart rate and pupil reactions. Moreover the myocardium could now be cut, or pinched with forceps, without sending it into fibrillation. Re-establishment of the circulation was much easier. There was a sense of tone about the animal. A further increase of flow to 200–225 ml./min. resulted in an animal indistinguishable from any normal animal, anaesthetized, and with its chest open. Thus it was shown that, on flows of from one-seventh to one-quarter of those given by other workers as the minimum for survival, a dog was able to exist, under a barbiturate anaesthetic, for 30 min. with its chest open, and make a perfect, immediate and long-term recovery.

a. Venous Return: Limiting Factor

As flows were increased above 150 ml./min., it became increasingly more difficult to recover blood from the femoral vein in sufficient quantity to maintain the flow through the heart. At 200–225 ml./min. the caval pool was quickly exhausted, and time had to be allowed for a re-accumulation to occur before the flow could be continued. No amount of suction on the femoral cannula produced any improvement of the return. Raising the head end of the dog did cause a temporary improvement, but persistence in this attitude led to death of the dogs from cerebral anaemia. The experiment served to demonstrate how poor vasomotor control was, even with other reflexes so markedly improved. There appears to be considerable pooling of blood in the tissue spaces under the conditions of experiment. Other factors contributing to poor venous return were possibly: position of dog on its back; low blood pressure; small circulating volume and hence very marked oxygen reduction in large tissue masses, resulting in capillary dilatation; open thorax and diaphragmatic insufficiency.

At 150 ml./min. the venous return could be maintained steadily for 30 min. However, it had been demonstrated that the venous return from the recipient would be the limiting factor to the input that could be demanded from the donor. Stokes & Gibbon (1950) pointed out that failure to obtain the venous return required to maintain the input accounted for many of the deaths in their series.

3. Donor-Circulation Experiments

Direct connexion of donor to recipient, without the intermediary control, resulted in rapid exsanguination of donor once the heart and lungs of recipient had been excluded. Little or no venous return could be obtained from recipient (Kerr, 1952; Andreasen & Watson, 1953a).

By crossing the input to, and the return from, the recipient through the same small pump, control of input and return at a rate commensurate with the obtainable venous return was secured. Continuous recording flow-meters in the input and return circuits permitted fairly accurate adjustment of the two flows, as well as the recording of any persistent discrepancy, which, after 30 min., might result in retention by the recipient of an excess of blood from the donor.

The most effective site for delivery of input was found to be at a point 1 cm. above the aortic valves; delivery higher up in the carotid, or further down in the aortic arch, resulted in a significant number of myocardial failures. The input was from donor carotid to recipient carotid; the return from recipient femoral vein to donor femoral vein. The relative weights of donor and recipient were chosen as near 15 kg. and 10 kg. as could be.

In actual donor-circulation experiments venous returns were significantly higher than in the azygos series, ranging from 220–280 ml./min. However, there were considerable fluctuations during an experiment. These had to be watched for and the input carefully adjusted from time to time to match the venous return. In early experiments, before sufficient control of this adjustment had been obtained, large venous debts were incurred in recipient, with obvious deterioration of donor as a consequence. By charting the flow at one-minute intervals, it was possible to calculate this venous debt and readjust it at the end of the experiment by passing the calculated quantity of blood from recipient back to donor. This pooling in recipient could amount to as much as 1,700 ml. in 30 min.; with experience it could be kept below 200 ml. over that period. As experience was gained, it was possible to have both dogs survive with regularity, and after some 50 such experiments it was considered justifiable to say that a recipient dog could be maintained by a controlled donor circulation during total exclusion of the heart and lung for 30 min.; and that both donor and recipient could be expected to recover completely from the experiment.

a. Experimental Intracardiac Surgery

With this amount of control of an animal whose heart and lungs had been excluded from the circulation, it was felt that

many of the problems of intracardiac surgery might now be approached with some possibility of solution. The first in order seemed to be that of actually opening the heart for a prolonged period, such as 30 min., with eventual recovery.

With this and other problems in view, and after a few trials, a preliminary series of 10 donor-circulation experiments was carried out in 1953, in which the donor's right ventricle was fully opened for periods of from 15 min. to 25 min. Full recovery was obtained in all donors; six recipients recovered; four others died through faulty technique, difficulties with venous return and other mishaps. One death only was directly due to fibrillation. Out of this work a number of technical problems were isolated for study: for example, method of suture of heart wall so that full normal distension of the chamber would be permitted without tearing out of the sutures, when the circulation was re-established; control of the drop of blood pressure in recipient when blood is sucked off too fast from the right atrium; the best method of collecting the blood spilled from the chamber and its walls, and returning this to the circulation, etc. Bahnson (private communication, 1954) of the Johns Hopkins Hospital, Baltimore, mentions a similar experience with blood sucked off from the right atrium. There are obvious problems connected with the physiology of donor circulation, and Cohen and his coworkers (Cohen & Lillehei, 1954a, 1954b; Cohen *et al.* 1954) have already taken up the study of some of the metabolic factors. They have obtained long-term survivals of animals on flows of the azygos factor range, by means of an ingenious autogenous lung oxygenator preparation.

b. Donor Circulation in Human Intracardiac Surgery

The only work on man is that of Lillehei and his colleagues at Minneapolis (their experimental work, already quoted above, is so accurate, clear-sighted and ingenious) but actual details of this are not available at the time of writing. Blalock (1954) mentions their success in at least three human cases, using the data obtained in the azygos and donor-circulation experiments on animals.

The large number of problems remaining in this field should not be ignored in the enthusiasm arising from the first successes in man. Any lasting advance can be made only at the expense of carefully regulated study; desire for success should not be permitted to hurry this. Too early and too eager application to human surgery of barely tested data can only retard progress and cause disappointment, quite apart from its being highly unethical.

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THE ARTIFICIAL HEART-LUNG AND ITS PRACTICAL APPLICATION TO CARDIAC SURGERY

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1 Development of technique
2 Clinical application
References and select bibliography

Further developments of cardiac surgery will largely depend on obtaining free access to the interior of the bloodless and motionless heart. This will mean that measures to safeguard the integrity of vital areas of the body must be effective and technically feasible. Either those areas must be artificially provided with sufficient oxygenated blood to meet their needs or the demand for oxygen must be so reduced that they can be excluded from the circulation for sufficient time to permit surgical correction of the cardiac abnormality. A third possibility is evident when these techniques are combined to the mutual advantage of each.

The purpose of this article is to discuss the present status of the first of these possibilities.

1. Development of Technique

In 1953 Gibbon (personal communication) reported the repair under direct vision of an interauricular septal defect—the patient, an adult, recovering completely. The normal circulation was excluded from the heart for 25 min., the heart's function and that of the lungs being imitated successfully by an artificial heart-lung machine. This report confirms that the results of similar operations on animals can be applied to man, and indicates that no insoluble problems are likely to hinder the eventual development of this technique of substitution of a mechanical system for the natural one. This development is now proceeding along two main channels.

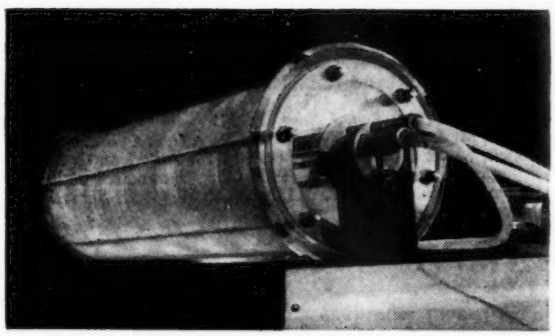
In one group are those who prefer a completely artificial system, and in the other are those who seek a substitute only for the heart—homologous lungs or even a human donor being used should oxygenation be required.

It is impossible to list the very many contributions to the literature, and a select bibliography as well as a list of references is included. However, certain papers are landmarks, and these include Gibbon (1937), Gibbon, Miller, Dobell, Engell & Voigt (1954), Björk (1948a, 1948b), Dennis (Dennis, Karlson, Eder, Nelson, Eddy & Sanderson, 1951; Dennis, Spreng, Nelson, Karlson, Nelson, Thomas, Eder & Varco, 1951) and Helmsworth, Clark, Kaplan & Sherman (1953) in the first group, and Andreasen & Watson (1953)[1], Dodrill, Hill, Gerisch & Johnson (1953), Mustard, Chute, Keith, Sirek, Rowe & Vlad (1954) and Warden, Cohen, Read & Lillehei (1954) in the second group.

When this problem was undertaken at the Postgraduate Medical School of London it was decided to concentrate on the completely artificial apparatus, though it was clear that very considerable problems were implicit in this. Partial by-pass of the heart by various short-circuit techniques is limited in scope, and the technique of cross-perfusion from a donor involves ethical considerations not easily solved. The use of homologous lungs, though an attractive possibility, was thought to present many of the problems associated with the transplanting of homologous tissue, as well as difficulties in availability, storage, and reliable function.

A solution to the problem of artificial oxygen and carbon dioxide exchange was first sought and that chosen has proved adequate in practice (Melrose, 1953). It is essentially a method of spreading blood in very thin films over a large surface and hence exposing the red cells directly to oxygen. The blood is passed through a large plastic cylinder (fig. 1) whose lumen is

FIG. 1. OXYGENATOR

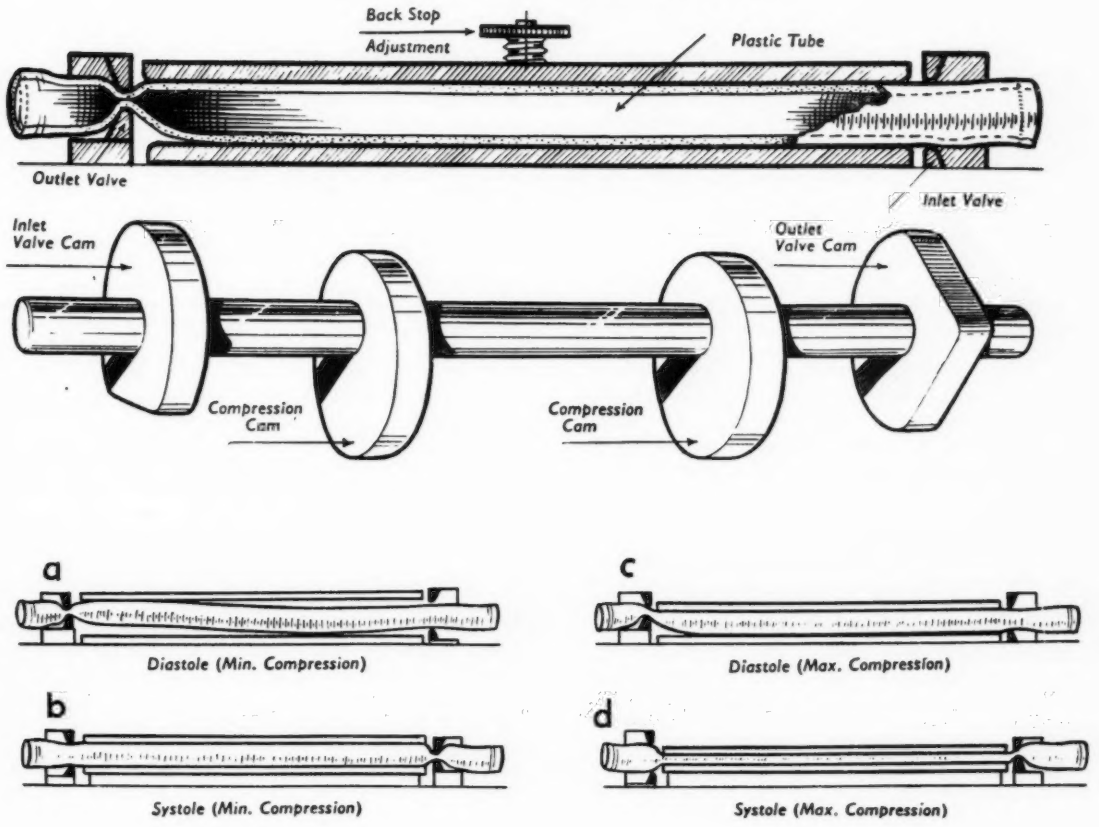


divided into a great number of compartments. These compartments are formed by an assembly of plates and washers in such a manner that the plates protrude into the lumen of the cylinder and in doing so provide a multitude of surfaces for oxygenation. This apparatus is at present constructed from Perspex which provides an inert surface on which little deposition of protein occurs. In action it is relatively atraumatic and it permits rapid blood flows with adequate gas exchange, allowing the normal adult oxygen consumption to be maintained.

Roller pumps were used in the original machine but these have now been replaced by pumps in which a straight plastic tube is squeezed between two metal plates (fig. 2). One plate acts as a back-stop against which the other plate compresses the tube. The pumping plate is driven by a camshaft which imparts an undulating movement to it. The back-stop can be moved towards or away from this moving plate and hence the amount of compression can be varied and with it the stroke volume. In practice a variation of output between zero and 5 l./min. can be obtained in this manner. Such a pump requires valves to direct the flow and, in order to maintain the

[1] See also Andreasen, p. 233 of this number of the Bulletin.—Ed.

FIG. 2. SCHEMATIC DIAGRAM OF PUMP



advantages of the roller pump in this respect, these valves do not lie within the blood stream. They are essentially miniature versions of the pumping plates, and completely occlude the tubing at appropriate points in the pumping cycle. This cycle provides a close imitation of the normal ventricular cycle.

The action of these pumps is considerably less traumatic than that of the roller pumps and it is safe to say that any danger from haemolysis has been eliminated by their use.

Experience has underlined the need for simplicity and reliability, and these considerations have encouraged the design of a mechanical system that will make the apparatus independent of electricity should the supply fail. For normal working an electronic control ensures that the blood volume in the machine is automatically maintained within narrow limits and that pressure limits are not exceeded. These aids can be overridden by the operator, who by rotating a crank-handle can work the entire apparatus.

There is little doubt that considerable improvements will occur as development continues, especially in the materials employed in such devices, and that many current difficulties will be solved. However, the real problems are likely to be those relating to actual application.

The most important of these lies in the fact that anticoagulants[2] are required in the operation of these extracorporeal circuits, and that during their use complicated alterations in the clotting mechanism take place which may render haemostasis extremely difficult.

This alteration is in part explicable by the development of a thrombocytopenia. The platelet count usually falls to low levels, and this drop may persist for some days. Three factors may produce a deficiency of coagulation components: (i) the operation itself—coagulation factors, especially platelets, are often reduced during major surgery ; (ii) a dilution of the recipient's blood by stored " bank " blood deficient in many clotting components and even actively productive of platelet deficiency ; (iii) the passage of blood over foreign surfaces which, though lined with silicone, still cause a marked loss of platelets by adhesion or destruction. At present there is no absolutely effective answer to this problem, though much can be done to offset the danger of persistent haemorrhage. The transfusion of fresh blood rich in platelets after the specific effect of the anticoagulant has been neutralized by such substances as protamine sulphate will restore the bleeding time and

[2] See Symposium on Blood Coagulation and Thrombosis, *Brit. med. Bull.* 1955, Vol. 11, No. 1.—ED.

allow normal wound closure. However, this is a fickle process and at best rather unspecific. A clearer understanding of the nature of the alteration in the blood will surely reveal a more direct treatment. An extensive survey of this important surgical problem has been made in J. H. Gibbon's unit at the Jefferson Medical College of Philadelphia, USA, and the studies there made by Bell and others (Bell, 1953) throw much light on it.

Surprising difficulties often attend the transference of well-tried experimental techniques to the clinical field, and methods of cannulation that are successful in healthy experimental animals may not always be appropriate. Venous blood must be gathered from the great veins themselves and access through peripheral vessels greatly limits the size of the cannulae used. This in turn limits the effective rate of perfusion and increases the destruction of cells because of the high velocity of flows along narrow tubes. The choice lies between multiple cannulae passed through peripheral veins into the major ones or a more direct approach through the right atrium. This latter technique allows a large-bore tube (8–15 mm.) to be accommodated and ensures efficient drainage at low suction pressures. However, the placing of such a cannula during procedures on the left heart, particularly when this is greatly enlarged, necessitates a wide exposure involving both sides of the chest with transection of the sternum. The advantages gained from this route must be balanced against the increased difficulty of haemostasis and closure of the wound, though it has been our experience that for efficient total by-pass of the heart every effort should be made to cannulate in this way.

The return of oxygenated blood into the arterial system does not present quite such a problem, but similar considerations govern the choice of entry. The use of narrow tubes, such as are required if the superficial femoral arteries are to be used, increases the rate of haemolysis, particularly if these tubes are long enough to reach the thoracic portion of the aorta. Yet it is important to reach such an area if the bulk of the returning blood is to be directed to the brain and myocardium. The artery of choice is undoubtedly the subclavian. No other vessel so easily carries the necessary flow, and a further asset is that it can be safely sacrificed if need be. This is particularly the case when small children are considered, when it may be the only route permitting a large enough cannula.

The observation of Andreasen & Watson (1953) that a dog would survive unharmed a prolonged period of minimal cardiac output, in fact one-tenth of the normal value, has led Lillehei (Warden, Cohen, Read & Lillehei, 1954) to use a much smaller blood flow than usual in many clinical perfusions. He claims that adequate support of the vital centres is provided by a blood flow of about half that predicted from the body-weight. This is an important point in that it may lead to much simplification of design of these machines and to an easing of the problems of cannulation.

The establishment of a satisfactory perfusion does not in itself ensure a " dry heart ". The coronary blood flow may exceed 500 ml./min., and a proportion of this will be carried by the Thebesian veins. Drainage of the right atrium and ventricle is necessary unless the coronary circulation is itself excluded by a clamp across the base of the aorta. This manoeuvre may be necessary where the aortic valves are so incompetent that they permit a large flow from the aorta to the left ventricle. For these two reasons it is important to assess the dangers and complications that attend exclusion of the coronary circulation. The ideal situation will be reached only when the surgeon is able to work on hearts from which all blood flow is excluded and which are quite inactive. Such a state may well be attainable for periods of at least 30 min. or longer. Present studies by one of us (D.G.M.) on the possibility of stopping all muscular activity for prolonged periods with full resumption of normal beating are encouraging and, should they be thoroughly substantiated, indicate that the ideal conditions may well be attainable.

Another advantage inherent in the " stopped heart " technique is the reduction in the danger of air embolism following open heart procedures. So great is this when surgery of the left heart is undertaken that special measures have to be taken to guard against it. Senning (1953) has suggested that it is safer in such circumstances to induce ventricular fibrillation, with the risk entailed of failure to restore normal beating, than to allow the ventricle to beat. Gibbon, Miller, Dobell, Engell & Voigt (1954) have overcome this danger experimentally by establishing a vent tube in the apex of the left ventricle and by applying suction to it to keep the ventricular cavity free of air. This greatly assisted them in a series of left ventriculotomies on dogs. Swan, Zeavin, Blount & Virtue (1953) have resorted to filling the chest cavity with Ringer's solution prior to cardiac closure to ensure that no air remains. Against this rather forbidding view of the dangers implicit in coronary artery air embolism—and it is the coronaries that are most affected—is the report of Geoghegan & Lam (1953) that 85% of dogs recovered from such air embolism following vigorous cardiac massage.

Leakage of blood through an incompetent aortic valve is not in practice so real a problem and has the advantage of preventing air embolism. The blood entering the ventricle can be effectively extracted and returned to the artificial circulation by a third pump. However, in one clinical instance such a leak was a hindrance, and unnecessarily large flows were required to maintain a useful pressure in the aorta.

2. Clinical Application

The use of the machine for clinical problems may take one of two forms: it may be used either to assist the circulation or to provide a complete cardiac by-pass.

In the former group the pump oxygenator acts as a support to an already defective heart, sufficient to enable closed surgical procedures to be carried out that under normal circumstances would not have been possible. Severe aortic stenosis provides an example of this group (Aird, 1954). Our experience with these cases, however, indicates that, although the assisted form of circulation makes surgical procedures possible, the myocardium is rarely able to shoulder the load of maintaining the circulation again when the pump is removed from the circulation.

Certain acute medical emergencies, such as coronary thrombosis, cor pulmonale, status asthmaticus and pulmonary embolism, may well be carried over a period of crisis by the use of an assisted circulation, but our experience in this field has been limited. If it is used in this way, without a surgical operation, other than that required to introduce the cannulae, the problems of incoagulable blood are no longer present and there is no real contra-indication to the use of the machine. We believe that its use in these cases is worthy of further trial.

The cardiac by-pass may take one of two forms, involving either the whole heart or one side only, though the difficulties of adequate cannulation are greater for the partial by-pass.

This method, however, does permit the performance of open surgical procedures on the heart, which is undoubtedly the ultimate aim of all these artificial procedures. Our experience in this field has been limited to cases of mitral incompetence and one of a ruptured aneurysm of a sinus of Valsalva into the right atrium and ventricle. A very satisfactory exposure of the mitral valve has been obtained and direct operative repair has been readily carried out.

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Notes on Contributors

Mr A. T. Andreasen, who is at present organizing and establishing full medical services for a group of important mines in Sierra Leone, has been Professor of Surgery at Bengal Medical College, Calcutta, India. He was made a Fellow of the Royal Society of Edinburgh in 1943, was Chairman of the Clinical Research Committee of the All-India Medical Research Fund Association from 1943 to 1948, and was Leverhulme Scholar and Streatfeild Scholar of the Royal College of Surgeons of England from 1949 to 1953. Mr Andreasen's research has included the following: a basis for classification of congenital heart defects; technical problems of chest surgery in dogs; the study of donor circulation as a means to elective intracardiac surgery; clinical and experimental studies on colitis and massive strangulation of the gut, as seen in tropical practice; and nutrition and shock amongst tropical peoples. Among his most important publications are papers on the education of a surgeon (*J. Indian med. Ass.* 1940, **9**, 512; **10**, 49); on experimental cardiovascular surgery (*Brit. J. Surg.* 1952, **39**, 548; 1953, **40**, 616; 1953, **41**, 195). He has also contributed two chapters to *Emergency surgery*, Pt 3 (1950) and Pt 5 (1953), by Hamilton Bailey.

Mr N. R. Barrett is Surgeon to St Thomas's Hospital, London, and to the Brompton Hospital, London. He is Consulting Thoracic Surgeon to the Royal Navy, to the Welsh National Memorial Association at Sully Hospital, to the King Edward VII Sanatorium at Midhurst and to the Ministry of Pensions and National Insurance. He spent a year in the USA in 1935 as a Rockefeller Travelling Fellow, and has lectured upon surgical subjects in the USA and most countries in western Europe. He delivered a Hunterian Lecture at the Royal College of Surgeons of England in 1955. He is the editor of *Thorax* and has made numerous contributions to medical journals, notably upon the surgical aspects of oesophageal disorders and hydatid disease of the lungs.

Sir Russell Brock has been Surgeon to Guy's Hospital, London, and to the Brompton Hospital, London, since 1936. He was formerly Demonstrator in Pathology, Demonstrator in Anatomy, and Surgical Registrar and Tutor at Guy's Hospital. From 1929 to 1930, he was a Rockefeller travelling fellow. He was awarded the Jacksonian Prize of the Royal College of Surgeons of England in 1935, was Hunterian Professor there in 1938, and became a member of the Council of the College in 1949. Sir Russell Brock's principal appointments have included those of Consulting Thoracic Surgeon to the London County Council from 1935 to 1946, Surgeon to the Ministry of Pensions from 1936 to 1945, and Thoracic Surgeon and Regional Adviser on Thoracic Surgery to the Emergency Medical Service from 1939 to 1946. In 1950, he was awarded the Julius Mickle Prize of London University; in 1953, the Fothergillian Gold Medal of the Medical Society of London and the Leriche Medal of the International Society of Surgeons; and in 1954, the Cameron Prize of Edinburgh University. Sir Russell Brock's publications include *Anatomy of the bronchial tree* (1946) and *Lung abscess* (1952), as well as numerous articles in surgical and medical journals.

Dr A. I. Parry Brown received his medical education at the London Hospital. After qualifying in 1931, he held various resident appointments before becoming Resident Anaesthetist at the London Hospital in 1934. In 1936 he was elected Assistant Honorary Anaesthetist to the London Hospital, eventually becoming Anaesthetist to the Department of Thoracic Surgery formed there by Mr Tudor Edwards in 1938. His interest in the problems of anaesthesia in thoracic surgery was confirmed by his subsequent appointment, under the Emergency Medical Service, to Mr T. Holmes Sellors' unit at Harefield Hospital where Mr Holmes Sellors' enthusiasm encouraged him to overcome, by use of posture, some of the difficulties of anaesthesia in thoracic surgery cases. This connexion with thoracic surgery brought him into contact with cardiac surgery as it developed. Dr Parry Brown's publications include a paper on posture in thoracic surgery (*Thorax*, 1948, **3**, 161), and, in collaboration with B. A. Sellick, on anaethesia for cardiac surgery (*Anaesthesia*, 1953, **8**, 4).

Dr Maurice Campbell is Physician in Charge of the Cardiac Department at Guy's Hospital, London, and Senior Physician to the National Heart Hospital, London. During the First World War, he served with the Royal Army Medical Corps in Mesopotamia and Persia, with the rank of captain, and was awarded the O.B.E. (Military Division). From 1924 to 1928, he was a Beit Memorial Research Fellow. Dr Campbell's published work includes papers on anaemia, goitre, heart disease, quinidine treatment, and, during recent years, especially on congenital heart disease.

Mr W. P. Cleland is Surgeon to the Brompton Hospital, London, Thoracic Surgeon to King's College Hospital, London, Lecturer in Thoracic Surgery at the Postgraduate Medical School of London, Civilian Consultant in Thoracic Surgery to the Royal Navy, and Thoracic Surgeon to the South-East and South-West Metropolitan Regional Hospital Boards. Mr Cleland received his training in thoracic surgery at the Brompton Hospital and at the Emergency Medical Service Hospital at Horton, Epsom, during the Second World War. His publications include chapters on lung abscess in *Diseases of the chest*, vol. 1 (1952), edited by G. Marshall & K. M. A. Perry, and on thoracic and intrathoracic injuries in *British surgical practice*, vol. 8 (1950), edited by E. Rock Carling & J. P. Ross.

Dr E. J. Delorme is a graduate of the University of Toronto Medical School and held an appointment there in 1938 as a demonstrator in anatomy and physiology. Since the Second World War he has been a travelling fellow of the Nuffield Foundation and of the Commonwealth Fund, and more recently held the Wilkie Scholarship in surgical research. He is at present a member of the External Scientific Staff of the Medical Research Council and Senior Lecturer in Experimental Surgery at the University of Edinburgh where he is engaged in full-time surgical research. He is the author of a paper on experimental arterial perfusion of the liver (*Lancet*, 1951, **1**, 259) and co-author of a number of papers on the use of radioactive isotopes in the study of the circulation. A preliminary communication on his work on hypothermia appeared in the *Lancet* (*Lancet*, 1952, **2**, 914), and the experimental study of this state has been his main interest since 1949.

Mr H. H. G. Eastcott was formerly Assistant Director of the Surgical Unit at St Mary's Hospital, London, and in association with Professor C. G. Rob has been responsible for developing practical methods of arterial grafting and for banking human arteries and corneas. As Consultant Surgeon to St Mary's Hospital, he is continuing this work, which was begun in 1949 in the Surgical Laboratory, Harvard Medical School, Boston, in collaboration with Dr Charles A. Hufnagel, now of Georgetown University, Washington. Arterial grafting was the subject of a Hunterian Lecture given by Mr Eastcott at the Royal College of Surgeons of England in 1953. His publications, with Professor C. G. Rob and others, include a description of artery banking by freeze-drying, and case reports on the use of hypothermia in major vascular surgery.

Mr Andrew Logan is Lecturer in Thoracic Surgery in the University of Edinburgh and Consultant in Thoracic Surgery in the South-Eastern Regional Hospital Board (Scotland). He came to Edinburgh in March 1946 from Newcastle upon Tyne, where he was Assistant Surgeon in the Royal Victoria Infirmary and Assistant Surgeon in the Newcastle General Hospital. During the Second World War he was Officer Commanding No. 1 Surgical Chest Team, Royal Army Medical Corps, and officer in charge of a surgical division in a general hospital. Some of Mr Logan's published papers, written in collaboration with R. W. D. Turner, are: "Mitral incompetence" (*Lancet*, 1952, **2**, 593); "Mitral stenosis" (*Lancet*, 1953, **1**, 1007); and "Aortic stenosis" (*Lancet*, 1954, **1**, 1091).

Dr I. K. R. McMillan received his medical training at the University of Cambridge and at St Thomas's Hospital Medical School, London. In 1946, he was appointed House Surgeon and Casualty Officer at St Thomas's Hospital, and subsequently was appointed House Surgeon at the Hospital for Sick Children, Great Ormond Street, London. After serving in the Royal Army Medical Corps, with the rank of major, from 1947 to 1950, Dr McMillan became Research Fellow in the Cardiac Department of St Thomas's Hospital from 1950 to 1951, and Mackenzie Mackinnon Research Fellow of the Royal College of Physicians and the Royal College of Surgeons of England from 1951 to 1954. From 1954 to 1955, he was Visiting Scientist at the National Heart Institute, Bethesda, Maryland. Dr McMillan's publications, with other authors, include: "Cardiac arrest and ventricular fibrillation" (*Thorax*, 1952, **7**, 205); "Aortic stenosis. A post-mortem cinephotographic study of valve action" (*Brit. Heart J.* 1955, **17**, 56); and "Some observations on blood gas and electrolyte changes during surface cooling" (*Ann. R. Coll. Surg. Engl.* 1955, **16**, 186).

Mr G. A. Mason is Lecturer in Thoracic Surgery at King's College, University of Durham, Senior Surgeon (in charge) to the Department of Thoracic Surgery at Shotley Bridge General Hospital, and Honorary Thoracic Surgeon to the Royal Victoria Infirmary, Newcastle upon Tyne. After graduation he held the Stephen Scott, Hamilton Drummond, and Heath Scholarships at the University of Durham. He was a Hunterian Professor at the Royal College of Surgeons of England in 1933, when he lectured on the surgical significance of the vitelline duct, and again in 1936, when the subject was "Extirpation of the lung". In the latter lecture, the two first successful pneumonectomies for bronchiectasis (both done in 1934) in England were recorded. Since then Mr Mason has been almost entirely engaged in thoracic surgical work. During recent years he has been principally concerned with major cardiovascular surgery and has lectured on this and other thoracic subjects in Norway, Germany, Holland, Spain and USA as well as in Great Britain. His publications include: "Cancer of the lung: review of a thousand cases" (*Lancet*, 1949, **2**, 587); "Myocardial ischaemia and its surgical relief" (*Lancet*, 1951, **1**, 358); and a chapter on surgery of the heart and great vessels in *Grey Turner's modern operative surgery*, vol. 1 (4th ed., 1955), edited by the late G. Grey Turner & L. C. Rogers.

NOTES ON CONTRIBUTORS

DR D. G. MELROSE studied medicine at Oxford University and at University College Hospital, London. After the usual junior appointments he became in 1952 an assistant lecturer in surgery at the Postgraduate Medical School of London and is now a lecturer there. Since 1949 he has studied the haemodynamics of the surgical patient and particularly the problems of cardiac surgery. During this time he has evolved a heart-lung machine capable of sustaining the circulation in man, and has explored the possibilities of general hypothermia. He has published various papers on these subjects, many of them the results of experiments carried out at the Buxton Browne Surgical Research Farm at Downe, Kent.

PROFESSOR C. G. ROB is Professor of Surgery in the University of London, and Surgeon to St Mary's Hospital, London. He is also a consultant in vascular surgery to the Army. He was formerly Surgeon to St Thomas's Hospital, London, and during the Second World War he was in the Royal Army Medical Corps as a lieutenant-colonel. He served as a surgeon in the First Parachute Brigade and was awarded the Military Cross. Professor Rob's work has been mainly concerned with general and peripheral vascular surgery, with particular reference to blood-vessel reconstruction, and also with the reconstruction of the cervical oesophagus and trachea. His present work is directed mainly at the further study of peripheral vascular disease and the use of plastic materials in bridging defects in arteries. He has also been studying for some time the use of hypothermia in patients, using external cooling. Professor Rob's most important publications include a paper on the preservation and transplantation of human tissues (*Lancet*, 1954, **2**, 255); a chapter on the reconstruction of the trachea, hypopharynx and cervical oesophagus, and, with H. H. G. Eastcott, a chapter on arterial grafting in *British surgical practice: surgical progress* (1951 and 1953), edited by E. Rock Carling & J. P. Ross.

MR D. N. ROSS is Senior Registrar in Thoracic Surgery at Guy's Hospital, London. After qualifying in Cape Town, he came to Great Britain on an E. Oliver Ashe Overseas Scholarship in 1946. He subsequently held appointments as Resident Surgical Officer and as Orthopaedic Registrar at the Royal United Hospital, Bath, and then in the Thoracic Surgical Unit of the Bristol Royal Hospital, where he became Senior Registrar in 1951. He was appointed Research Fellow in Cardiovascular Surgery at Guy's Hospital in 1952. Mr Ross's research has included work on storage and chemical sterilization of aortic homografts, the physiological investigation of hypothermia, and the development of apparatus and techniques of cooling for use in man. His present work includes the clinical management of cardiac and thoracic surgical cases and, in the experimental field, continued work on hypothermia, particularly in the lower temperature ranges, and the investigation of cross-circulation and the exploration of its clinical possibilities. Mr Ross's publications include papers on the technique and physiological investigations of hypothermia (*Guy's Hosp. Rep.* 1954, **103**, 97); on venous cooling (*Lancet*, 1954, **1**, 1108); and, with Sir Russell Brock, on the clinical application of hypothermic techniques (*Guy's Hosp. Rep.* 1955, **104**, 99).

DR B. A. SELLICK received his medical education at the Middlesex Hospital Medical School, London, and has since held resident appointments there. During the Second World War, he served as Specialist in Anaesthesia in the Royal Navy in the Far East, and, on demobilization in 1946, he was appointed Honorary Anaesthetist to the Middlesex Hospital. He is now Consultant Anaesthetist to the Middlesex Hospital and to the Thoracic Surgical Unit at Harefield Hospital, and Lecturer to the Faculty of Anaesthetists of the Royal College of Surgeons, to which he was elected a Fellow in 1953. Dr Sellick visited Copenhagen during a recent poliomyelitis epidemic and reported to the Ministry of Health on methods of artificial ventilation in respiratory paralysis. He is now a member of the Poliomyelitis Advisory Committee of the South-West Metropolitan Regional Hospital Board. Over the past nine years, he has made a study of anaesthetic methods for thoracic surgical procedures in general and for cardiac surgery in particular. In 1953, he visited several centres in Canada and the USA with this end in view. He has also carried out clinical investigations into post-operative pulmonary complications. His present work at the Institute of Clinical Research of the Middlesex Hospital Medical School includes experiments on hypothermia and on the control of cardiac irregularity during cardiac surgery. Dr Sellick's most important publications, with other authors, include: "Effect of procaine penicillin and breathing exercises in postoperative complications" (*Lancet*, 1952, **1**, 345); "The prevention of postoperative pulmonary atelectasis" (*Lancet*, 1953, **1**, 164); and "Anaesthesia for cardiac surgery" (*Anaesthesia*, 1953, **8**, 4).

MR T. HOLMES SELLORS received most of his training in general surgery at the Middlesex Hospital, London, where he held the post of Surgical Registrar. He was the first George Herbert Hunt Travelling Scholar and spent some months studying thoracic work in Scandinavia. He is at present Surgeon to the London Chest Hospital, an appointment he has held since 1934, and to Harefield Hospital, Harefield, Middlesex. In 1947, he was appointed Thoracic Surgeon to the Middlesex Hospital, where a great deal of cardiovascular surgery is now carried out. His past appointments include Assistant Surgeon to Queen Mary's Hospital, London, and to the Royal Waterloo Hospital, London. At the outbreak of the Second World War he was appointed Regional Adviser in Thoracic Surgery to the Emergency Medical Service, and in 1944 he was Hunterian Professor of the Royal College of Surgeons of England. He has been awarded La Médaille de la Reconnaissance Française, and is an Officer of the Order of Carlos Finlay. He is also a member of numerous foreign surgical societies, and has lectured abroad for the British Council. Mr Holmes Sellors has published numerous articles on thoracic surgery, including "Valvotomy in the treatment of mitral stenosis", with D. E. Bedford & W. Somerville (*Brit. med. J.* 1953, **2**, 1059), and "Results of surgical treatment of carcinoma of the lung" (*Brit. med. J.* 1955, **1**, 445); he is also editor, with J. L. Livingstone, of *Modern practice in tuberculosis* (1952), to which he has contributed several chapters.

MR O. S. TUBBS is Thoracic Surgeon to St Bartholomew's Hospital, London, and Surgeon to the Brompton Hospital, London. He received his training as a surgeon at St Bartholomew's Hospital and subsequently as a thoracic surgeon both at St Bartholomew's Hospital and at the Brompton Hospital. From 1937 to 1938 he was with Dr R. H. Overholt in Boston, and it was while he was there that his interest in the possibilities of cardiac surgery was stimulated by Dr Strieder's attempt to close an infected patent ductus. On returning to Great Britain, Mr Tubbs closed an infected patent ductus in December 1939, with a successful result, and his interest in cardiac surgery has continued from that time. Mr Tubbs' most important publications include: "Ligation and chemotherapy for infection of patent ductus arteriosus" in collaboration with G. Bourne & K. D. Keele (*Lancet*, 1941, **2**, 444); "The effect of ligation on infection of the patent ductus arteriosus" (*Brit. J. Surg.* 1944, **32**, 1); and a chapter on chronic constrictive pericarditis in *British surgical practice: surgical progress* (1953), edited by E. Rock Carling & J. P. Ross.

DR PAUL WOOD is Director of the Institute of Cardiology, and Physician to the National Heart Hospital, London. He is also Physician in Charge of the Cardiac Department, Brompton Hospital, London. In 1941 he delivered the Goulstonian Lecture, in 1950 the St Cyres Lecture, and in 1954 the Strickland Goodall Lecture. He is author of *Diseases of the heart and circulation* (1950) and of various papers which have appeared in the medical press. An article by Dr Wood, entitled "Pulmonary hypertension", appeared in *British Medical Bulletin*, 1952, **8**, 348.

* * *

Book Review

Garrison and Morton's Medical Bibliography: An Annotated Check-List of Texts Illustrating the History of Medicine

Leslie T. Morton. 2nd ed. London: Grafton & Co., 1954. xiii + 655 pages. 22 × 14 cm. £5 5s.

Medical historians—and many others of us who make no claim to that high status—will be pleased to see a new edition of the well-established favourite, "Garrison and Morton", for which the second-named author is again responsible. In the Introduction he points out that the whole work has been completely overhauled, and some 1,400 new entries added; the total number of items now reaches the impressive figure of 6,808.

Under each subject dealt with, the important works which show the development of that subject are arranged in chronological order, and it is the author's object to cite the first known medical description of specific diseases; but the uninformed reader must not suppose that the first such description necessarily represents the earliest unequivocal mention of the disease in question. It might be of general interest to digress and give some instances: In Old Irish-Gaelic there were two names used for smallpox, each descriptive of an outstanding characteristic of the infection—*bolgach* i.e. "pock-disease", and *clamtrusca* which described the later stage of heavy scabbing. The first word is still in common use; the other has been forgotten for more than three centuries, but its meaning is unquestionable. Thus, the old scholar Conall MaGeoghagan when translating into Elizabethan English rendered *clamtrusca* by "pox" in one entry; and in another entry he explains that in Gaelic the English "pox" is called *bolgach*—a double check on the meaning of the word, if this were in any doubt.

In the ancient annals, the earliest record of an epidemic of smallpox that I have found under the first of these names is dated A.D. 577, and under the second, A.D. 552[1], that is, both of them more than 300 years before Rhazes' account of the disease. It is to be noted that these old annalists were not writing medical tracts; they were recording great events, and only a widespread and killing epidemic would qualify for mention in chronicles of general history. And the only epidemic disease of this character that either of these names could possibly describe, is smallpox.

To take another example of a disease that was recognized long before the earliest known medical description of it: The first account of poliomyelitis that the author cites is Underwood's "Debility of the lower extremities" in 1789. But more than a thousand years before this date, there was recognized—also in Ireland—an epidemic disorder that caused paralysis, and in consequence was called *baccach*, the lameness. The word implies weakness or paralysis affecting any of the limbs: at one time the English word "lameness" had the same wide meaning. An outbreak of "The plague that is called the lameness" is recorded in A.D. 707; such a description of a spreading infection could apply only to poliomyelitis, and the annalist's phrase "that is called" suggests that it was already familiar at that period. So far as I can discover, the annals include no mention of this malady other than the one in question; but, as I have pointed out (*Irish Historical Studies*, 1949, **6**, 169), this may have been the only occasion on which the factors necessary for such a record, conjoined—an outbreak intense enough to reach the annalistic grade of a "plague", the eye to see it, and the hand to note it down. The initial febrile phase of the infection would pass the understanding of any scribe of old, but no one could fail to observe the trail of cripples that it left behind.

Of course, no criticism is implied in the foregoing remarks, for the type of references I give, and this only for their general interest, does not come within the author's purpose. All the same, in so wide a field it is inevitable that sometimes he should fall short of his expressed aim. Thus, he says that Rutty's account of the fever, other than typhus, in the Irish famine of 1741, is "the first clear description of relapsing fever". But compare Rutty's very brief—though clear—description with the much fuller one in the Hippocratic Collection, *Epidemics*, **1**, 20–22:

> "The great majority had a crisis on the sixth day, with an intermission of six days followed by a crisis on the fifth day after the relapse. Those who had a crisis on the seventh day had an intermission of seven days, with a crisis on the third day after the relapse", etc.
>
> "Most had rigors near the first crisis, and those who had rigors at first near the crisis, had rigors again in the relapse at the time of the crisis." The rigors happened "by far the most during the winter." Jaundice and profuse epistaxis, the commonest complications of louse-borne relapsing fever, are both mentioned.

It was the first of these two complications that gave the disease its former name "The yellow fever", used not only in common speech but by medical writers—e.g. Lind, Stokes, and Graves—as well. And there can be no doubt that the great epidemic of the mid 6th-century, called "The yellow plague" in Latin, Gaelic, and Welsh alike, was the same disease.

This new edition is assured of a warm and a wide welcome, and readers of every category will be especially grateful to the author for the admirable little annotations which define the scope of so many of the works that he includes here.

W. P. Mac Arthur

[1] In another MS., 554. Such differences in dates sometimes arose from errors in copying; sometimes from miscalculations in the mathematical feat of turning the ancient Irish calendar of 84-year cycles distinguished by lunar and ferial data, into the newer A.D. notation.

* * *

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British Medical Bulletin

Surgery of the Heart and Thoracic Blood Vessels

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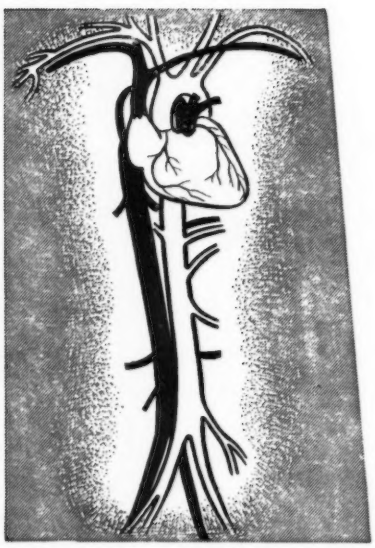

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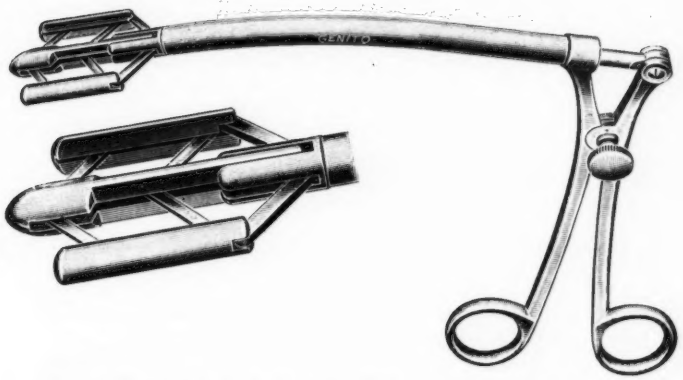

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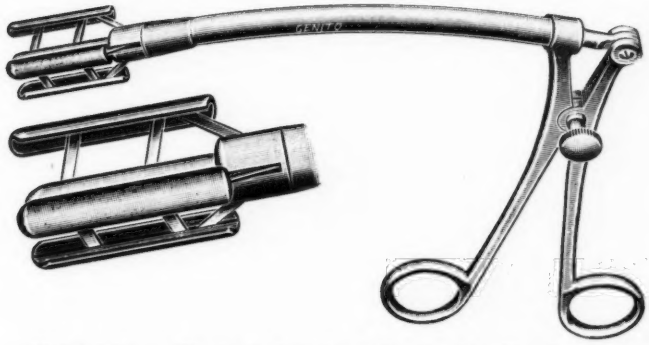

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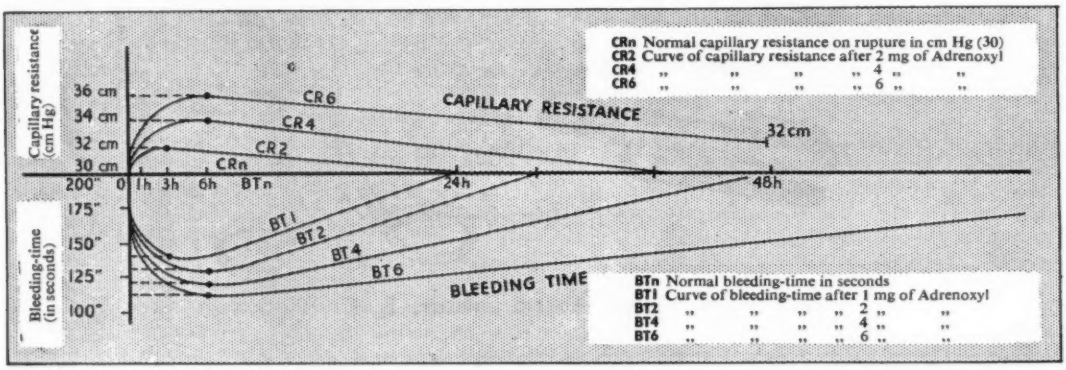

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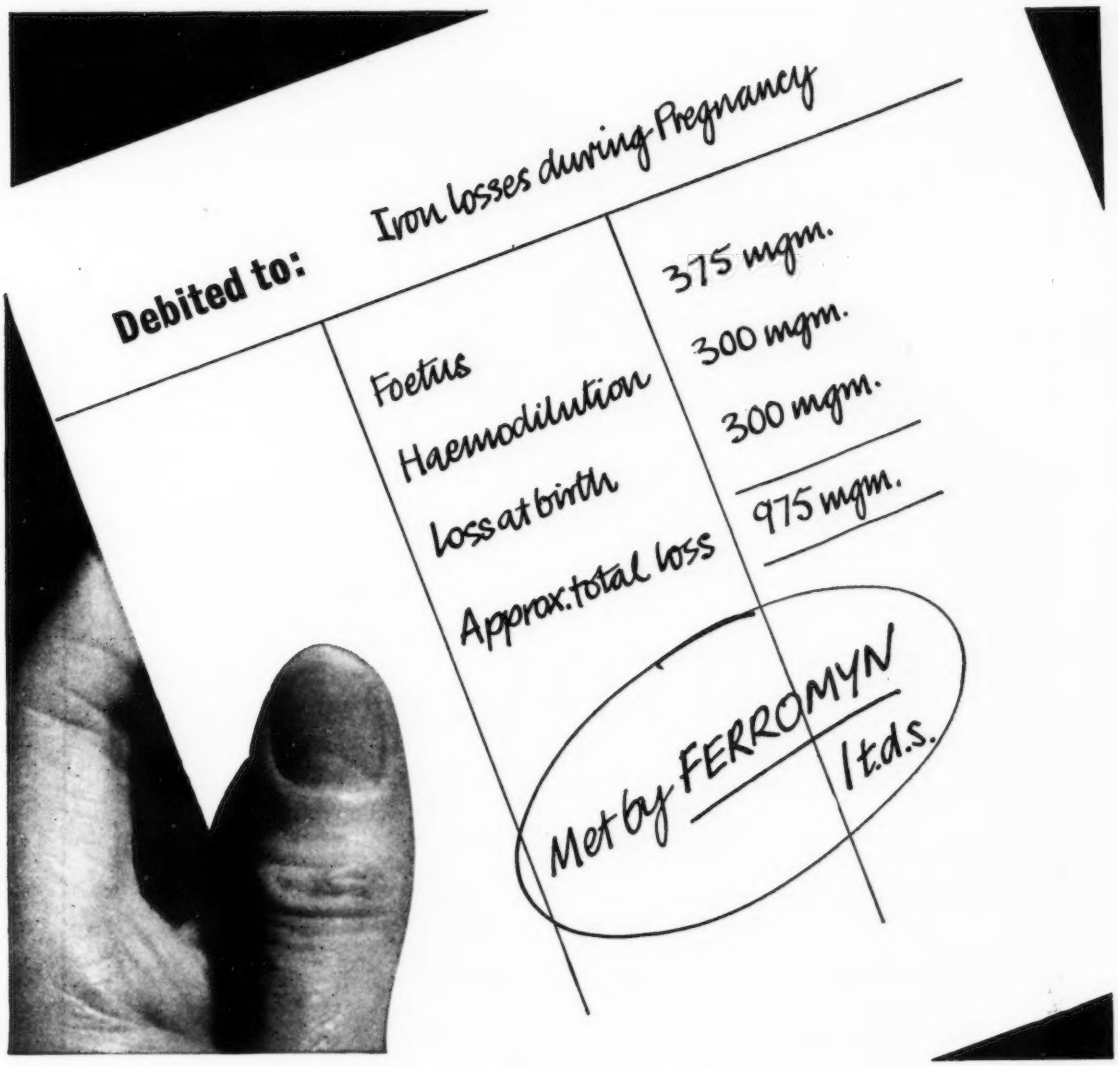

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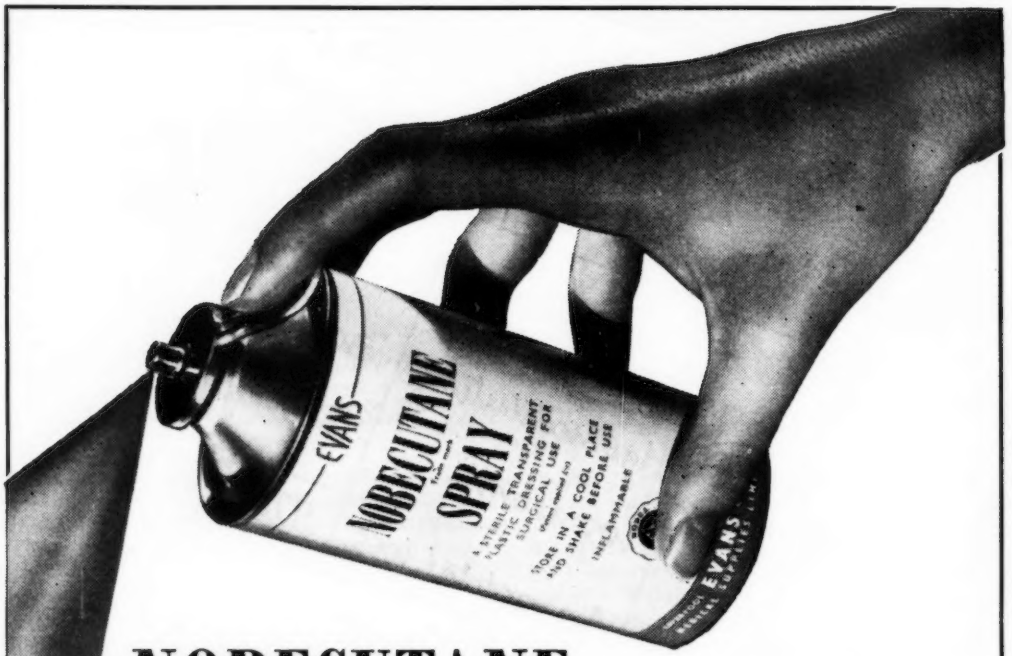

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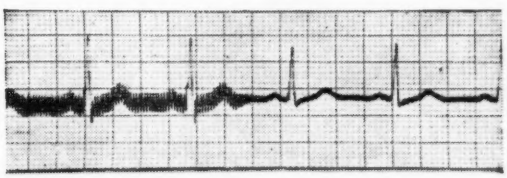

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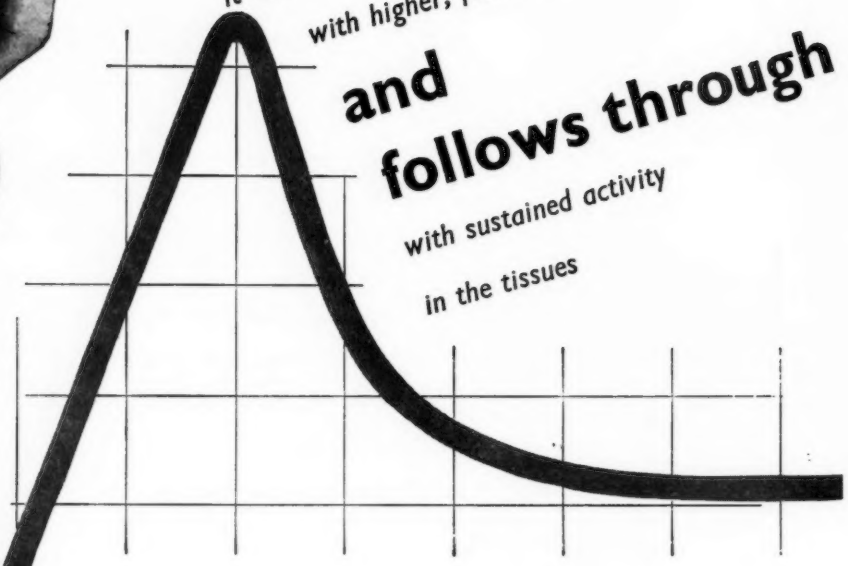

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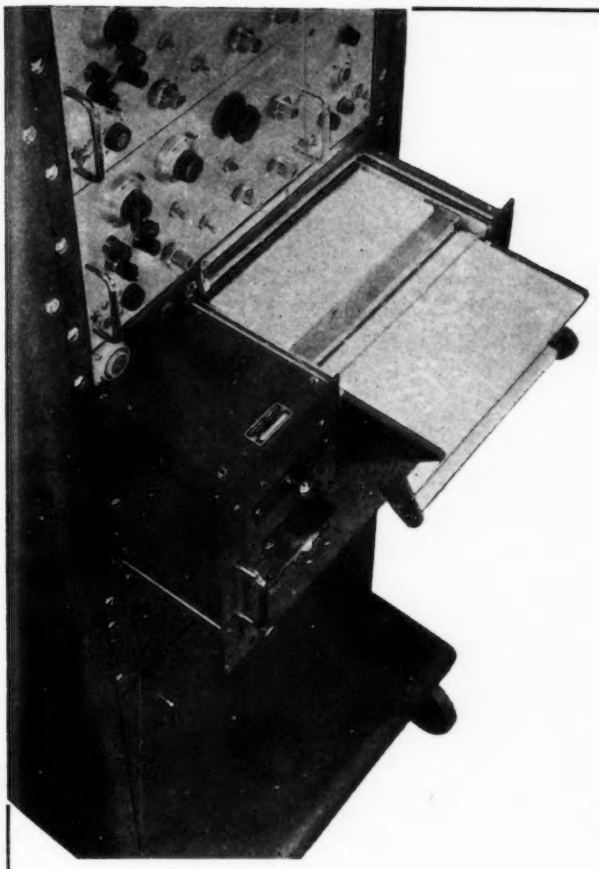

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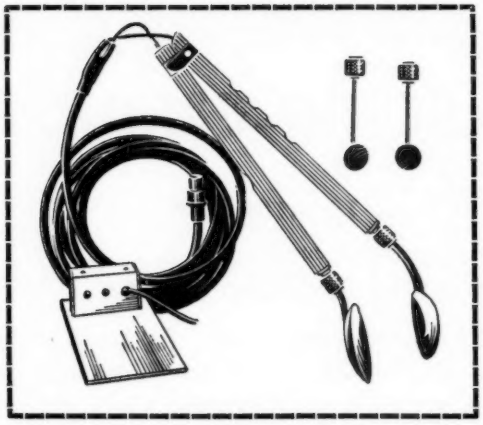

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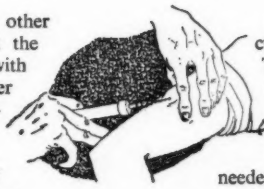

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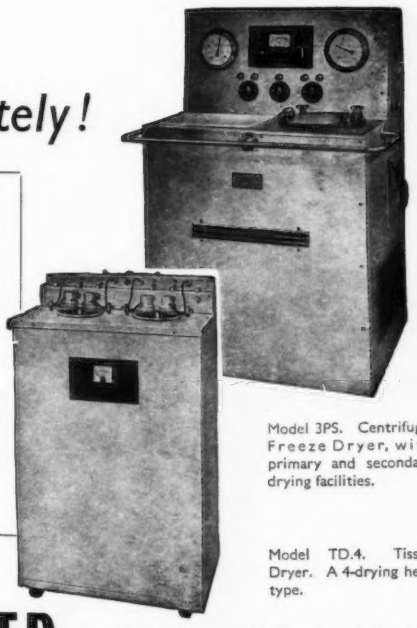

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